Red Cell 2,3-Diphosphoglycerate and Oxygen Affinity of Hemoglobin in Patients With Thyroid Disorders

By Charles G. Zaroulis, Ione A. Kourides, and C. Robert Valeri

We measured red blood cell 2,3-diphosphoglycerate (2,3-DPG), adenosine triphosphate (ATP), and the P50 value in vitro of the oxyhemoglobin dissociation curve, which is the oxygen tension at half saturation of hemoglobin, in order to quantitate red blood cell oxygen transport function in individuals who were diagnosed as hypothyroid, euthyroid, or hyperthyroid based on measurements of thyroxine (T4), triiodothyronine (T3), thyrotropin (TSH), and their clinical status. Hypothyroid (mean T4 2.8 µg/dl, T3 49 ng/dl, TSH 37 µU/ml) and hyperthyroid (mean T4 14 µg/dl, T3 271 ng/dl, TSH <0.7 µU/ml) patients had normal red cell 2,3-DPG and ATP levels and normal P50 values in vitro. The known changes in oxygen consumption produced by alterations in thyroid hormone levels in patients with hypothyroidism or hyperthyroidism did not affect red blood cell oxygen transport function.

It has been shown that 2,3-diphosphoglycerate (2,3-DPG) is one of the most important ligands involved in the facilitated release of oxygen from free hemoglobin (Hb) in solution or in intact red blood cells. Adenosine triphosphate (ATP), another red blood cell organic phosphate compound, has also been shown to facilitate dissociation of oxygen from Hb.

Gahlenbeck and Bartels suggested that hyperthyroid patients have decreased red blood cell oxygen affinity for Hb. They showed that euthyroid humans and rats have decreased oxygen affinity of Hb in the blood after treatment with triiodothyronine (T3) and suggested that this reduction was caused by the increased metabolic demands of the induced hyperthyroid state. Several publications have reported increases in red blood cell 2,3-DPG in hyperthyroid individuals as well as in normal human red blood cells after incubation in vitro with various concentrations of thyroid hormones, although there was no significant correlation between thyroid hormones and the red cell 2,3-DPG level. An increase in 2,3-DPG was observed in an incubation system in vitro when a purified enzyme preparation of 2,3-DPG mutase was treated with T3 and thyroxine (T4). Other investigators have failed to observe an increase in 2,3-DPG by incubation in vitro of human red blood cells or purified 2,3-DPG mutase enzyme with thyroid hormones. Contrary to what would be expected, in hypothyroid patients who have a reduced oxygen requirement, decreased red blood cell 2,3-DPG levels and increased oxygen affinity of red blood cells were not observed.

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Table 1. Hematologic and Thyroid Function Data in Patients With Thyroid Disorders and Euthyroid Controls

<table>
<thead>
<tr>
<th>Patients</th>
<th>Hb (g/dl)</th>
<th>Hct (%)</th>
<th>MCHC (g/dl RBC)</th>
<th>P50 Value In Vitro (mm Hg)</th>
<th>Serum Inorganic Phosphorus (mg/dl)</th>
<th>RBC 2,3-DPG (μmol/g Hb)</th>
<th>RBC ATP (μmol/g Hb)</th>
<th>T4 (μg/dl)</th>
<th>T3 (μg/dl)</th>
<th>Serum TSH (mU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal range</td>
<td>12.5–15.5</td>
<td>38–48</td>
<td>31–35</td>
<td>29–31</td>
<td>3.0–4.5</td>
<td>12.5–15.0</td>
<td>3.5–4.5</td>
<td>4–11</td>
<td>70–170</td>
<td>0.2–3.2</td>
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<tr>
<td>Euthyroid controls</td>
<td>Mean ± 1 SE</td>
<td>13.0 ± 0.5</td>
<td>41 ± 1.5</td>
<td>33.6 ± 0.5</td>
<td>30.0 ± 1.3</td>
<td>3.3 ± 0.3</td>
<td>14.4 ± 0.8</td>
<td>3.6 ± 0.2</td>
<td>6.1 ± 0.5</td>
<td>76 ± 5</td>
</tr>
<tr>
<td>N</td>
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<tr>
<td>Hypothyroid</td>
<td>Mean ± 1 SE</td>
<td>12.8 ± 0.4</td>
<td>40 ± 1.2</td>
<td>32.7 ± 0.5</td>
<td>31.8 ± 1.0</td>
<td>3.7 ± 0.2</td>
<td>14.1 ± 0.6</td>
<td>4.1 ± 0.2</td>
<td>2.8 ± 0.3*</td>
<td>49 ± 6*</td>
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<tr>
<td>Hyperthyroid</td>
<td>Mean ± 1 SE</td>
<td>13.4 ± 0.6</td>
<td>41 ± 1.5</td>
<td>33.1 ± 0.3</td>
<td>30.7 ± 1.0</td>
<td>3.9 ± 0.4</td>
<td>14.7 ± 0.7</td>
<td>4.2 ± 0.3</td>
<td>14 ± 1*</td>
<td>271 ± 39*</td>
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*Significantly different from euthyroid by unpaired t test, p < 0.05.
†All hyperthyroid individuals had undetectable serum TSH concentrations, whereas TSH was measurable in all but one euthyroid control subject.
Since there have been investigations of red blood cell 2,3-DPG levels and red blood cell affinity for oxygen in few patients with thyroid disorders, we studied hypothyroid patients, hyperthyroid patients, and euthyroid normal individuals in order to evaluate the utility of measurements of red blood cell 2,3-DPG and ATP levels and $P_{50}$ values in vitro as a sensitive indicator of the metabolic effects of thyroid hormones.

**MATERIALS AND METHODS**

**Patients.** Twenty-three subjects were studied after giving informed consent for these studies: nine hypothyroid patients (seven females, two males) studied on 14 occasions, seven female euthyroid controls studied on 9 occasions, and seven hyperthyroid patients (six females, one male) studied on 9 occasions. One of the hypothyroid patients received incremental doses of levothyroxine ($L-T_4$) at monthly intervals over a 5-mo period with correction of her hypothyroid state. This patient was studied monthly throughout her treatment schedule.

**Assays.** Measurements of Hb, microhematocrit, mean corpuscular hemoglobin concentration (MCHC), serum inorganic phosphorus, red cell $P_{50}$ value in vitro of the oxyhemoglobin dissociation curve (the oxygen tension at 50%, saturation of Hb for washed red cells maintained at pH 7.2, pCO$_2$ 0, and 37°C), red blood cell 2,3-DPG, and red blood cell ATP were performed as previously described. Total $T_4$, total $T_3$, and thyrotropin (TSH) concentrations were also measured by previously reported methodology.

**RESULTS**

The euthyroid, hypothyroid, and hyperthyroid subjects had normal hematologic data; there were no significant differences in the Hb, hematocrit, MCHC, serum inorganic phosphorus, red cell $P_{50}$ in vitro, ATP, or 2,3-DPG values among the euthyroid control, hypothyroid, or hyperthyroid groups (Table 1). However, significant differences in thyroid function were observed among the groups chemically as well as clinically. Red blood cell 2,3-DPG and TSH were not significantly related in the subjects with detectable TSH values (hypothyroid and euthyroid control groups); the hyperthyroid group was not included because of undetectable TSH levels. In addition, there was no significant correlation between TSH levels and the $P_{50}$ in vitro or ATP levels. There was no significant correlation between the red cell 2,3-DPG level or $P_{50}$ value in vitro and serum $T_4$.

One hypothyroid patient (TSH 113 µU/ml, $T_4$ 2.0 µg/dl, $T_3$ 88 ng/dl) was treated with increasing daily doses of $L-T_4$ at monthly intervals: she did not show any significant change in red cell 2,3-DPG or $P_{50}$ value in vitro throughout the course of replacement therapy, although on 300 µg $L-T_4$ daily she was chemically hyperthyroid (TSH 0.8 µU/ml, $T_4$ 14.5 µg/dl, $T_3$ 150 ng/dl) (Fig. 1).

**DISCUSSION**

In previous studies involving only a small number of patients, it was suggested that the level of 2,3-DPG was higher in hyperthyroid patients than in normal individuals. The differences in results may have been due to the small number of patients in the previous studies, the methods used to measure thyroid function, or the methods used to evaluate the response to therapy.

Oxygen transport to tissues is influenced by blood flow, pulmonary function, red cell volume, and the red cell affinity for oxygen. Oxygen requirements of tissues are influenced by body temperature and thyroid function. The pa-
Fig. 1. Red blood cell 2,3-DPG content, $P_{50}$ value in vitro, and $T_3$, $T_4$, and TSH levels in a female with primary hypothyroidism prior to and during a 5-mo period of treatment with L-thyroxine.

Patients studied had chronic hyperthyroidism or hypothyroidism of a severe degree without anemia or any apparent pulmonary dysfunction, although no patient was so decompensated as to be considered in myxedema coma or thyroid storm. Alteration in cardiac function in our patients may well have been sufficient to compensate for the altered oxygen requirements. Cardiac output has been shown to vary directly with thyroid status. Moreover, recent reports have shown that left ventricular contractility changes directly with thyroid function. Nevertheless, it is possible that certain patients with hyperthyroidism and decreased cardiac reserve may require increased levels of 2,3-DPG and decreased affinity for oxygen to meet the increased peripheral tissue demands for oxygen. We conclude that measurements of red blood cell 2,3-DPG and ATP levels or the $P_{50}$ value in vitro do not serve as sensitive indicators of the tissue effect of thyroid hormones in most patients with thyroid dysfunction.

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