# Hemoglobin Properties of Egyptian Obese Smokers:
## Effect of Combination of Obesity and Smoking

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**Abstract:** The present investigation was designed to throw light on the effect of smoking coupled with obesity in human on the Hemoglobin oxygen affinity that plays an important role in enabling Hemoglobin carrying out its functions normally. Thirty-seven healthy white Egyptian men between the ages of 25 and 35 years, live in the same area, a small farm near Helwan, Cairo participated in this study. Nine nonsmoker men were chosen to represent the control group. Twenty eight men who smoked one or more packs of cigarettes per day constituted the study group after divided into two subgroups obese and non-obese. Erythrocytes count, different hemoglobin derivatives and hemoglobin oxygen affinity were measured. Smokers group individuals were chosen depending on smoking rate, those with a rate less than one packet per day were excluded. All individuals are exposed to measuring the different hemoglobin derivatives and those with elevated results were chosen for carrying out the experiment. Results showed that obese smokers recorded the highest concentration of abnormal hemoglobin derivatives and the lowest oxygen affinity, i e their hemoglobin has the lowest chance to carry oxygen to and from lung and muscles due to decrement of the hemoglobin active sites able to bind with oxygen and carbon dioxide. It is concluded that the hazardous of smoking reach its peak in the obese more than non-obese smokers.

**Key words:** hemoglobin properties, obese smokers, hemoglobin derivatives

## INTRODUCTION

In most humans, the hemoglobin molecule is an assembly of four globular protein subunits. Each subunit is composed of a protein chain tightly associated with a non-protein heme group. Each protein chain arranges into a set of alpha-helix structural segments connected together in a globin fold arrangement, so called because this arrangement is the same folding motif used in other heme/globin proteins such as myoglobin (Steinberg, 2001). This folding pattern contains a pocket which strongly binds the heme group (Hardison, 1996).

A heme group consists of an iron (Fe) ion (charged atom) held in a heterocyclic ring, known as a porphyrin. The iron ion, which is the site of oxygen binding, bonds with the four nitrogens in the center of the ring, which all lie in one plane. The iron is also bound strongly to the globular protein via the imidazole ring of the F8 histidine residue below the porphyrin ring. A sixth position can reversibly bind oxygen, completing the octahedral group of six ligands. Oxygen binds in an "end-on bent" geometry where one oxygen atom binds Fe and the other protrudes at an angle. When oxygen is not bound, a very weakly bonded water molecule fills the site, forming a distorted octahedron (Weber and Vinogradov, 2001).

The iron ion may either be in the $Fe^{2+}$ or $Fe^{3+}$ state, but ferrihemoglobin (methemoglobin) ($Fe^{3+}$) cannot bind oxygen. In binding, oxygen temporarily oxidizes ($Fe^{2+}$) to ($Fe^{3+}$), so iron must exist in the +2 oxidation state in order to bind oxygen. The enzyme methemoglobin reductase reactivates hemoglobin found in the inactive ($Fe^{3+}$) state by reducing the iron center (Sioban, *et al.,* 2008).

In the tetrameric form of normal adult hemoglobin, the binding of oxygen is thus a cooperative process. The binding affinity of hemoglobin for oxygen is increased by the oxygen saturation of the molecule (Imai, *et al.,* 1989), with the first oxygen bound influencing the shape of the binding sites for the next oxygen, in a way favorable for binding. This positive cooperative binding is achieved through steric conformational changes of the hemoglobin protein complex, i e when one subunit protein in hemoglobin becomes oxygenated, this induces a conformational or structural change in the whole complex, causing the other subunits to gain

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an increased affinity for oxygen (Hardison, 1996). As a consequence, the oxygen binding curve of hemoglobin is sigmoidal, or S-shaped, as opposed to the normal hyperbolic curve associated with noncooperative binding (Maillett, *et al.,* 2008).

The three-dimensional structure of hemoglobin is best described as a pair of identical $\alpha\beta$ dimers ($\alpha_1\beta_1$ and $\alpha_2\beta_2$) that associate to form the hemoglobin tetramer. In deoxyhemoglobin, these $\alpha\beta$ dimers are linked by an extensive interface, which includes, among other regions, the carboxyl terminus of each chain. The heme groups are well separated in the tetramer with iron-iron distances ranging from 24 to 40 Å. The deoxy form corresponds to the T state in the context of either the concerted or the sequential model for hemoglobin cooperativity. On oxygen binding, there are substantial changes in quaternary structure that correspond to the T-to-R state transition (Vitagliano, *et al.,* 2008).

Hemoglobin's oxygen-binding capacity is decreased in the presence of carbon monoxide because both gases compete for the same binding sites on hemoglobin, carbon monoxide binding preferentially in place of oxygen (Perego, *et al.,* 1996). Carbon dioxide occupies a different binding site on the hemoglobin (Slaughter and Lanc, 1983). Carbon dioxide is more readily dissolved in deoxygenated blood, facilitating its removal from the body after the oxygen has been released to tissues undergoing metabolism. This increased affinity for carbon dioxide by the venous blood is known as the Haldane effect. Through the enzyme carbonic anhydrase, carbon dioxide reacts with water to give carbonic acid, which decomposes into bicarbonate and protons (Kneipp, *et al.,* 2005).

The current study aimed to evaluate the effect of the combination between smoking and obesity on the ability of Hemoglobin to carry out its normal function specially the oxygen affinity.

## MATERIALS AND METHODS

### Subjects:

Thirty-seven healthy Egyptian men between the ages of 25 and 35 years, live in the same area, at a small farm near Helwan, Cairo, participated in this study. Nine nonsmoker's men were chosen to represent the control group. Twenty eight men who smoked one or more packs of cigarettes per day constituted the study group after divided into two subgroups obese (n=14) and non-obese (n=14). No subject had donated blood during the 6 months prior to study. Smokers group individuals were chosen depending on smoking rate, those with a rate less than one pack per day were excluded. All individuals are exposed to measuring the different hemoglobin derivatives and those with elevated results were chosen for carrying out the experiment.

### Procedures:

The following studies were done on the same venous blood sample collected from each subject on the morning of the study: blood counts and the concentration of different Hb derivatives. Hemoglobin oxygen affinity was measured in vivo before collecting the blood sample.

Red blood cells count (RBCs), mean corpuscular volume (MCV) and hemoglobin concentration were determined by Erythrocyte Mean Corpuscular Test, NCI C0369183, to avoid anemic effects hence in some types of anemia some hemoglobin derivatives are normally decreased.

### Oxygen-affinity of hemoglobin:

Oxygen affinity ($SpO_2$) was measured by using Fukuda Denshi, SD 7100 system, which measures the functional oxygen percentage that expressed as a fraction of the total amount of hemoglobin capable of transporting oxygen. By utilizing the light of the two wavelengths DS 71 00 can analyze for both oxygenated and deoxygenated hemoglobin and consequently can determine the functional $SpO_2$ (Kaka, 1992).

### Determination of Hemoglobin Derivatives Concentrations:

The millimolar extinction coefficients were put into four linear equations with the four unknown concentrations of hemoglobin pigments ($C_{HbO2}$, $C_{HbCO}$, $C_{MetHb}$ and $C_{SHb}$).

$$A^{500} = 5.05\,C_{Hbo2} + 5.35\,C_{HbCO} + 9.04\,C_{Met.Hb} + 7.2\,C_{SHb} \tag{1}$$

$$A^{569} = 11.27\,C_{Hbo2} + 14.27\,C_{HbCO} + 4.1\,C_{Met.Hb} + 8.1\,C_{SHb} \tag{2}$$

$$A^{577} = 15.37\,C_{Hbo2} + 10.0\,C_{HbCO} + 4.1\,C_{Met.Hb} + 8.1\,C_{SHb} \tag{3}$$

$$A^{620} = 0.24\,C_{Hbo2} + 0.33\,C_{HbCO} + 3.35\,C_{Met.Hb} + 20.8\,C_{SHb} \tag{4}$$

Where the absorption bands at wavelengths 500, 569, 577 and 620 nm represent the absorption maxima of Met-Hb, HbCO, $HbO_2$ and SHb, respectively.

This linear system of equations was solved by mathematical manipulation, using the Gaussian elimination method. For matrix calculation (Blum, 1972). to yield the following equations:

$$C_{SHb} = \frac{A^{620} - 0.442293 A^{500} + 0.1065519 A^{569} + 0.0515769 A^{577}}{18.895404} \tag{5}$$

$$C_{Met.Hb} = \frac{9.0602343 A^{500} - A^{577} - 2.6960235 A^{569} - 35.295898\, C_{SHb}}{66.750821} \tag{6}$$

$$C_{HbCO} = \frac{A^{569} - 2.2316831 A^{500} + 16.074415\, C_{Met.Hb} + 7.9681188\, C_{SHb}}{2.330495} \tag{7}$$

$$C_{HbO_2} = \frac{A^{500} - 5.35\, C_{HbCO} - 9.04\, C_{Met.Hb} - 7.2\, C_{SHb}}{5.05} \tag{8}$$

Where $A^{500}$, $A^{569}$, $A^{577}$ and $A^{620}$ are the absorbances of hemoglobin solution at the wavelengths 500, 569, 577 and 620 nm, respectively.

### *Statistical Analyses:*

Data were presented as means ± SD. A computer program (SPSS 9.0, SPSS Inc. Chicago, IL) was used for statistical analysis. A one-way ANOVA test was applied to data to detect significant differences between groups. Differences were considered significant at $P < 0.05$, and highly significant at $P < 0.01$.

## RESULTS AND DISCUSSION

### *Results:*

Results in Table 1 showed that RBCs count decreased in either obese and non-obese smokers as compared to non-smokers controls. The same trend was recorded in MCV and Hb concentration. Results of non-obese smokers are better than those obtained from obese smokers.

A significant decrease in oxy-hemoglobin concentration was recorded in both groups of the smoking individuals. On the other hand, all abnormal hemoglobin derivatives concentrations showed a highly significant increase when compared with control, table 2.

**Table 1:** RBCs count, MCV and hemoglobin concentration in relation to smoking status and body condition (Mean ± SD)

| Group | Red Blood Count (million/μL) | MCV (fl) | Hemoglobin (g/dl) |
|---|---|---|---|
| Nonsmoker (N = 9) | 5.3 ± 0.34 | 87.23 ± 8.45 | 14.3 ± 0.92 |
| Smokers Obese (N = 14) | 4.1 ± 0.29 | 76.76 ± 7.22 * | 12.9 ± 0.89 ** |
| Non-obese (N = 14) | 4.8 ± 0.31 | 86.17 ± 7.22 * | 12.5 ± 0.56 ** |

*P< 0.05   ** P< 0.01

**Table 2:** Different hemoglobin derivatives concentration in relation to smoking status and body condition (Mean ± SD)

| Group | S- Hb | Met-Hb | Hb-CO | $HbO_2$ |
|---|---|---|---|---|
| Nonsmoker (N = 9) | 0.665 ± 0.03 | 1.81 ± 0.52 | 3.916 ± 0.086 | 93.07 ± 0.27 |
| Smokers Obese (N = 14) | 0.786 ± 0.034 * | 2.271 ± 0.261** | 4.93 ± 0.327** | 88.23 ± 0.219** |
| Non-obese (N = 14) | 0.693 ± 0.043 * | 2.074 ± 0.257** | 4.44 ± 0.764* | 91.26 ± 0.242** |

*P< 0.05   ** P< 0.01

**Table 3:** Hemoglobin Oxygen affinity of the smokers group compared with the nonsmokers group.

| Group | $SpO_2$ |
|---|---|
| Nonsmoker (N = 9) | 98.34 % |
| Smokers Obese (N = 14) | 94.71 % ** |
| Non-obese (N = 14) | 95.57 % ** |

** P< 0.01

Oxygen affinity in both groups of smokers showed highly significant decrease, the lowest value was recorded in obese smoker individuals as compared to control, table 3. Oxygen saturation curve of smokers group either obese or non-obese is shifted to left. Higher shifting is recorded for obese smoker individuals, Fig. 1.
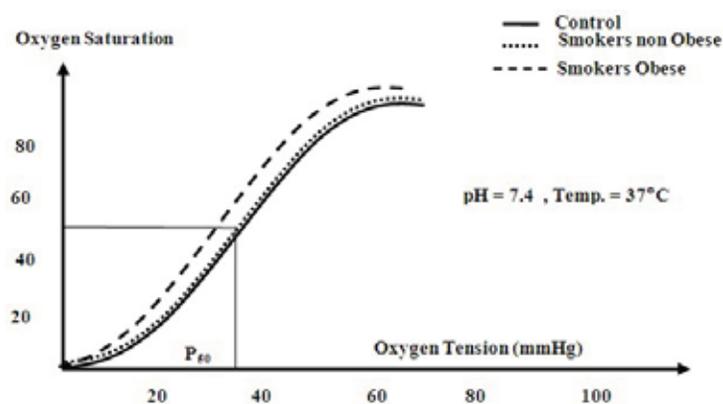
**Fig. 1:** Oxygen saturation curve of smokers groups compared with non smokers.

***Discussion:***

A small amount of molecular oxygen ($O_2$) is dissolved in blood, whereas the majority (98 %) is bound to haemoglobin[Steinberg , 2001]. Hemoglobin, the $O_2$ transport molecule in the blood, comprises four subunits: two α and two non α (e.g. β, γ or δ) subunits. Each subunit contains seven helices and a porphyrin heme iron moiety. It is heme iron that reversibly binds $O_2$. The heme iron can exist in the $Fe^{2+}$ or $Fe^{3+}$ oxidation state[Couture,*et al.,* 1999]. Only hemoglobin molecule contains ferrous heme can carry oxygen. This is called the tetramer referring to the four proteins subunits, each one made of 141 and 146 amino acids residues, respectively. This denoted as α2β2. The subunits are structurally similar and about the same size. Each subunits has a molecular weight of about 17000 Daltons for a total molecular weight 68000 Daltons (Imai, *et al.,* 1989).

In normal mammalians approximately 98 % of haemoglobin molecules have iron in the ferrous state that is suitable for carrying oxygen and carbon dioxide to and from lungs. In some haematological disorders some of the normal haemoglobin form (oxyhemoglobin) is converted to a nonfunctional ferrihemoglobin derivatives (methemoglobin, sulphohemoglobin and carboxyhemoglobin). Hemoglobin's primary structure is to bind oxygen that diffuse into the blood stream from the lungs and then transport it to outlying tissues where it is released primarily for aerobic respiration. Hemoglobin has the capacity to bind one and four oxygen molecules (Clara, *et al.,* 1995).

The dynamics of oxygen exchange is highly regulated by several metabolically-derived factors that collectively define the "oxygen demand" of an individual's tissues. Among the key metabolic factors regulating the dynamics of hemoglobin's oxygen exchange reactions is oxygen itself. When oxygen levels are high, the capacity of a partially saturated hemoglobin molecule to bind oxygen disproportionately increases with the number of oxygen molecules, it has already bound. In other words, when environmental oxygen levels are high, partially saturated hemoglobin molecules exhibit enhanced affinity for binding additional oxygen molecules, a specialized behavior referred to as cooperativity. Equally important, hemoglobin also manifests cooperativity in the reverse direction: When environmental oxygen levels are low, hemoglobin's affinity for oxygen drops disproportionately as fewer and fewer oxygen molecules remain to bind to hemoglobin. Thus, the cooperative loading or unloading of oxygen from hemoglobin, depending on the environmental concentration of oxygen, effectively enhances the oxygen uptake and delivery capacity of hemoglobin (Weber and Vinogradov, 2001).

This work was designed to find out the inter-relation between changes in the normal ratios of different hemoglobin derivatives may be imbalanced due to smoking and the overall oxygen affinity of the individual hemoglobin in obese and non obese objects.

RBCs count, MCV and Hb concentration were carried out just to exclude anemic objects to avoid the conflict that may stem on a decrease on the oxygen affinity not due to a decrease in the affinity phenomenon itself but due to a decrease in the hemoglobin amount.

Different Hemoglobin derivatives measurements showed significant increase of abnormal hemoglobin derivatives in smokers either obese or non-obese as compared to controls. Obese subjects recorded higher abnormal hemoglobin derivatives with comparing to non obese subjects. On the other hand, oxyhemoglobin concentration which is the normal form of hemoglobin was found to be decrease in smokers as compared to control and it showed the lowest one in obese subjects. This finding may be explained as a decrease in the hemoglobin property that enabling it to carry oxygen without reacting with it. After the oxygen reached to the

iron atom, oxidation process is occurred, so the iron is converted to the higher state $Fe^{3+}$ that unable to bind with other molecular oxygen and carbon dioxide and as a result these abnormal hemoglobin derivatives (S-Hb, Met-Hb and Hb-CO) were formed.

Results recorded for obese smokers group showed a significant increase in the abnormal forms of hemoglobin as compared to either control or non-obese smokers group, this finding may be interpreted as the highly storage adipose tissue in obese patients may act as a storage area for smoking toxins that play a role in the oxidation process of hemoglobin forming different hemoglobin derivatives. This findings in agreement with the curve plotted in the oxygen affinity experiments (Benesch, *et al,* 1965).

The sigmoid shape appears in Fig. (1) that represent the oxygen dissociation curve (ODC) comes from the interaction between the four globin moieties of the hemoglobin molecule and the molecular oxygen. In the beginning of the interaction oxygen is conjugated to one globin moiety easily due to excess of active sites to which oxygen can bind to. Then, hemoglobin oxygen affinity has to be increased in the second, third and fourth interaction as a result of the decrease in these active sites. In other wards oxygen affinity becomes at its peak when the chances of binding between oxygen and globin protein is reduced (Weber and Vinogradov, 2001, Arthur, *et al.,* 1973). Here, we can find out the main problem in obese-smokers hence they already suffering from an increase in the abnormal hemoglobin derivatives those have distortion in the intrastructure. So, the arrangement of the active sites on the globin surface is reduced as compared to normal. The final result of this process is such hemoglobin will not able to increase its oxygen affinity as the above mentioned interaction between oxygen and globin may has to occur (Hellerstedt, *et al.,* 1997).

Increased fractions of COHb, S-Hb and MetHb are dangerous for two reasons: (a) these subunits inhibit $O_2$ transport by blocking heme iron-binding sites; and (b) when one or more iron atoms has bound carbon monoxide or been oxidized, the hemoglobin conformation is changed so that the $O_2$ affinity of the remaining heme groups is increased, thus shifting the ODC to the left and decreasing $O_2$ delivery to tissues (Clara,*et al.,* 1995). A very important note has to be considered, all hemoglobin derivatives are measured by the routine laboratorial methods depending on converting all Hemoglobin derivatives found to cyanomethemoglobin after interaction with cyanide compounds and then determined by using spectrophotometer. In these methods all forms of hemoglobin able or not able to bind with oxygen were calculated, which is a very noticeable concentration in some cases as results obtained in our study.

In conclusion, combination between smoking and obesity has a great hazardous in the human health regarding their hemoglobin interaction with oxygen. These persons may suffer from a lot of general health problems that may appear by time.

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