Hemoglobin Oxygen Affinity of Egyptian Obese Smokers Suffering from Elevated Non-Functional Hemoglobin Derivatives

M.R. Bassem, W.A. Samir and Nahed Saleh

Department of Biochemistry, National Research Centre, Dokki, Giza, Egypt

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 to 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 oxygen affinity · 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 [1]. This folding pattern contains a pocket which strongly binds the heme group [2].

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 [3].

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 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 [4].

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 [5], with the first oxygen bound influencing the shape of the binding sites for the next oxygen, in a way favourable 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 an increased affinity for oxygen

Corresponding Author: Dr. M.R. Bassem, Department of Biochemistry, National Research Centre, Dokki, Giza, Egypt

[2]. 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 [6].

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 [5].

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 [7]. Carbon dioxide occupies a different binding site on the hemoglobin [8]. 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 [9].

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 packet per day were excluded. All individuals are exposed to measuring the different hemoglobin derivatives and those with elevated results were chosen to 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 cell count, mean corpuscular volume (MCV) and hemoglobin concentration are 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) is 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 7100 can analyze for both oxygenated and deoxygenated hemoglobin and consequently can determine the functional SpO_2 [10].

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}), by application in the Beer-Lamber law we obtain:

$$Eq. A^{500} = 5.05\, C_{HbO_2} + 5.35\, C_{HbCO} + 9.04\, C_{Met.Hb} + 7.2\, C_{SHb}$$

(1)

$$Eq. A^{569} = 11.27\, C_{HbO_2} + 14.27\, C_{HbCO} + 4.1\, C_{Met.Hb} + 8.1\, C_{SHb}$$

(2)

$$Eq. A^{577} = 15.37\, C_{HbO_2} + 10.0\, C_{HbCO} + 4.1\, C_{Met.Hb} + 8.1\, C_{SHb}$$

(3)

$$Eq. A^{620} = 0.24\, C_{HbO_2} + 0.33\, C_{HbCO} + 3.35\, C_{Met.Hb} + 20.8\, C_{SHb}$$

(4)

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

This linear system of equations was solved by mathematical manipulation, using the Gaussian elimination method. For matrix calculation [11] to yield the following equations:

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

(6)

$$Eq. \, C_{Met.Hb} = \frac{9.0602343 A^{500} - A^{577} - 2.6960235 A^{569} - 35.295898 \, C_{SHb}}{66.750821}$$
(7)

$$Eq. \, C_{HbCO} = \frac{A^{569} - 2.2316831 A^{500} + 16.074415 \, C_{Met.Hb} + 7.9681188 \, C_{SHb}}{2.330495}$$
(8)

$$Eq. \, C_{HbO_2} = \frac{A^{500} - 5.35 \, C_{HbCO} - 9.04 \, C_{Met.Hb} - 7.2 \, C_{SHb}}{5.05}$$
(9)

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.

Data were analyzed statistically by application of t-test.

RESULTS

Results in Table 1 showed that RBC count decrease in either obese and non-obese smokers as compared to non-smoking 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 is recorded in both groups of the smoking individuals. On the other hand, all abnormal hemoglobin derivatives concentration showed a highly significant increase.

Table 1: RBC 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**
Smokers non-obese (N= 14)	4.8±0.31	86.17±7.22	12.5±0.56**

*P < 0.05** P < 0.01

Oxygen affinity in both groups of smokers showed highly decrease. The lowest value was recorded in obese smoker individuals.

Oxygen saturation curve of smoker group either obese or non-obese is shifted to left. Higher shifting is recorded to obese smoking individuals.

DISCUSSION

A small amount of molecular oxygen (O_2) is dissolved in blood, whereas the majority (98 %) is bound to Hemoglobin [1]. 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_{II} or Fe_{III} oxidation state [12]. Only Hemoglobin molecule contains ferrous heme can carry oxygen. This is called the tetramer referring to the four protein subunits, each one made of 141 and 146 amino acids residues, respectively. This denoted as $\alpha_2\beta_2$. 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 [5].

In normal mammalians approximately 98 % of Hemoglobin 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 Hemoglobin form (oxyhemoglobin) is converted to afunction ferrihemoglobin derivatives (methemoglobin, sulphemoglobin 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 [13].

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

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

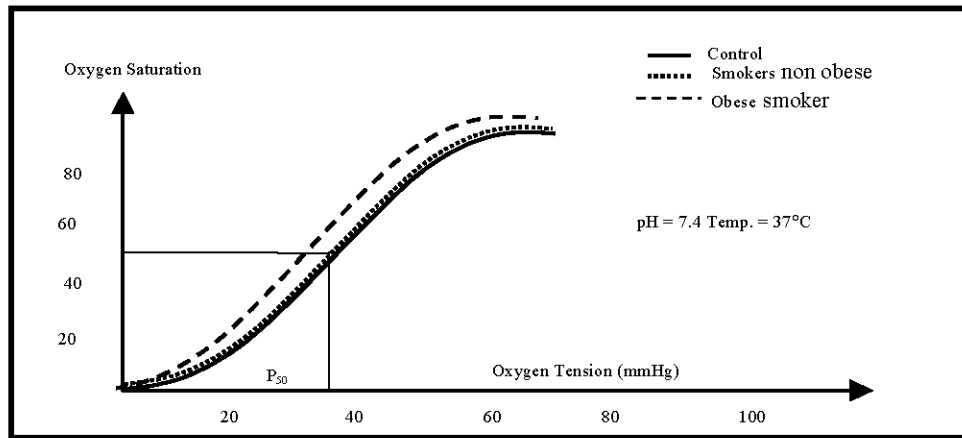| Group	S- Hb	Met-Hb	Hb-CO	HbO₂
Nonsmoker (N=9)	0.665±0.03	1.81±0.52	3.916±0.086	93.07±0.27
Smokers Obese (N=14)	0.786±0.034*	2.271±0.261**	4.93±0.327**	88.23±0.219**
Smokers Non-obese (N=14)	0.693±0.043 *	2.074±0.257**	4.44±0.764*	91.26±0.242**

*P< 0.05** P<0.01

Fig. 1: Oxygen saturation curve of smokers groups compared with non smokers

Table 3: Hemoglobin oxygen affinity in relation to smoking status and body condition (Mean ± SD)

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

*P< 0.05** P<0.01

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 behaviour 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 [3].

This work was designed to find out the inter-relation between changes in the normal ratios of different Hemoglobin derivatives 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 normal controls. Obese subjects recorded higher abnormal Hemoglobin derivatives with compared to non obese subjects. On the other hand, oxyhemoglobin concentration which is the normal form of Hemoglobin is decreased 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 finding is in agreement with the curve plotted in the oxygen affinity experiments [14].

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 hmoglobin 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 [3,15]. 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 infrastructure. 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 [16].

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 [13].

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.

REFERENCES

1. Steinberg, M.H., 2001. Disorders of Hemoglobin: Genetics, Pathophysiology and Clinical Management, Cambridge University Press.
2, Hardison, R.C., 1996. "A brief history of hemoglobins: plant, animal, protist and bacteria", Proceeding of National Academic Science, USA.
3. Weber, R.E. and S.N. Vinogradov, 2001. "Nonvertebrate hemoglobins: functions and molecular adaptations". Physiological. Review, 81: 569-628.

4. Sioban, B., T. Keel, G. Yang, J. Quigley, J. Chen, S. Knoblaugh, P.D. Kingsley, I.D. Domenico, B. Michael, J. Kaplan, J. Palis and J.L. Abkowitz, 2008. A Heme Export Protein Is Required for Red Blood Cell Differentiation and Iron Homeostasis. Science, 319: 825-828.
5. Imai, K., A. Tsuneshige, T. Harano and K. Harano, 1989. Structure-function relationships in hemoglobin Kariya, Lys-40(C5) alpha- ---Glu, with high oxygen affinity. Functional role of the salt bridge between Lys-40 alpha and the beta chain COOH terminus. Journal of Biological Chemistry, 264: 11174-11180.
6. Steinberg, M.H., 2001. Disorders of Hemoglobin: Genetics, Pathophysiology and Clinical Management, Cambridge University Press,
7. Perego, G.B., G.C. Marenzi, M. Guazzi, P. Sganzerla, E. Assanelli, P. Palermo, B. Conconi, G. Lauri and P.G. Agostoni, 1996. Contribution of PO_2, P50 and Hb to changes in arteriovenous O_2 content during exercise in heart failure. Journal of Applied Physiology, 80: 623.
8. Slaughter, R.L. and R.A. Lanc, 1983. Theophylline clearance in obese patients in relation to smoking and congestive heart failure. Annual Pharmacotherpeutics, 17: 274-276.
9. Kneipp, J., G. Balakrishnan, R. Shen, T.J. Sahu, N.T. HO, J.L. Giovannelli, V. Simplaceanu, C. HO, and T.G. Spiro, 2005. Dynamics of allostery in hemoglobin: roles of the penultimate tyrosine H bonds". Journal of Molecular Biology. 17: 335-53.
10. Kafka, D., 1992. Heart potential mapping from the thorasic surface in healthy person. Pollet Archeival Medical Wemn. 88: 127-40.
11. Blum, E.K., 1972. Numerical analysis and computation theory and practice. Addison - Wesley publishing company, Massachusetts, Menlo park, California. London. Don Mills, Ontario,
12. Couture, M., T.K. Das, H.C. Lee, J. Peisach, D.L. Rousseau, B.A. Wittenberg, J.B. Wittenberg and M. Guertin, 1999. Chlamydomonas chloroplast ferrous hemoglobin. Heme pocket structure and reactions with ligands. Journal of Biological Chemistry. 274: 6898-6910.
13. Clara Fronticelli, M.T. Sanna, G.C. Perez-Alvarado, M. Karavitis, A. LU and W.S. Brinigar, 1995. Allosteric Modulation by Tertiary Structure in Mammalian Hemoglobins. Journal of Biological. Chemistry, 270: 30588-30594.

14. Benesch, R, C. Macduff and R. Benesch, 1965. Determination of oxygen equilibrium with a new versatile tonometer. Analytical Biochemistry, 11: 81-508-511.

15. Arthur, L., J.R. Sagone, T. Lawrence and S.P. Balcerzak, 1973. Effect of smoking on tissue oxygen supply. Blood, 41: 845-851.

16. Hellerstedt, W.L., J.H. Himes, M. Story, I.R. Alton and L.E. Edwards, 1997. The effects of cigarette smoking and gestational weight change on birth outcomes in obese and normal-weight women. American Journal of Public Health, 87: 591-596.