Arch. Biol. Sci., Belgrade, 65 (3), 911-917, 2013 DOI:10.2298/ABS1303911B

GLYCATED HEMOGLOBIN IN CAMEL: MINIMAL CORRELATION WITH BLOOD GLUCOSE LEVEL

MOHAMMAD D. BAZZI, MUSHARI QUAZANI, NAYYAR RABBANI*, AJAMALUDDIN MALIK, MOHAMMED AL HASAN, MOHAMMED S. ELROBH and ABDULRAHMAN M. AL SENAIDY

Protein Research Chair, Department of Biochemistry, College of Science, King Saud University, Riyadh, KSA

Abstract - Glucose and glycated hemoglobin (Hb) in the blood of camel (*Camelus dromedarius*) and cow (*Bos taurus*) were analyzed and compared with human values. Camel displayed high blood glucose concentration (9.7±2.8 mM) but a low level of glycated-Hb (3.4± 0.23%).Cow blood samples did not show sufficient variations in glucose concentrations (5.7±0.73 mM) or glycated Hb levels (3.2± 0.11%) compared to human values. The low glycation of camel Hb at higher glucose concentrations suggest that certain factors protect the Hb from glycation at high glucose concentrations. Camel Hb also exhibited a higher electrophoretic mobility than normal hemoglobin of human or cow. Camel Hb migrated at a rate corresponding to that of human Hb-C. Bioinformatics tools were used to explore the biochemical basis for the difference in camel Hb migratory position and its apparent resistance to glycation.

Key words: Blood, camel, glucose, glycation, hemoglobin, modeling

INTRODUCTION

The Arabian camel is important in the Arabian and African deserts for economical and cultural reasons. The camel has many interesting physiological adaptations that enable it to survive the harsh desert environment (Bogin, 2000; Gaughan, 2011; Ouajd and Kamel, 2009). This fact has promoted several investigations to determine the "normal" values of several biochemical parameters in the camel, including blood enzymes, hematological parameters, blood metabolites, and others. There are considerable discrepancies in the reported values, possibly due to differences in the genetic, nutritional, and environmental conditions of the animal (Al-Rehaimi et al., 1989; Amin et al., 2007; Barakat and Abdel-Fattah, 1971; Bogin, 2000; Kataria et al., 2002; Mohamed and Hussein, 1999; Ouajd and Kamel, 2009).

This paper focuses on two aspects of camel hemoglobin, the universal oxygen carrier in the blood of all living organisms, its glycation status and electrophoric mobility. Glycation proceeds via the non-enzymatic reaction of glucose with proteins, including Hb, resulting in the formation of glycation adducts (Bunn, 1981; Peacock, 1984). Several reports suggest that camel blood has normal to high concentrations of glucose compared to other animals and human (Al-Ali et al., 1988; Ali et al., 2006; Mohamed and Hussein, 1999; Nazifi et al., 1998; Yagil and Berlyne, 1977). However, no attempts have been made to correlate the extent of Hb glycation with blood glucose concentration in the camel.

The present study reveals that the normal blood glucose concentration in the camel is significantly higher than in human blood: despite this, camel Hb showed a lower degree of glycation. The mobility of

camel Hb observed in capillary electrophoresis was consistent with human HbC, an inherited variant of HbA.

MATERIALS AND METHODS

Blood samples were collected from 11 camels and 5 cows in tubes containing Na_2EDTA (10mg/ml of blood) as an anticoagulant, from a slaughterhouse in the Riyadh area, Saudi Arabia. Blood samples were placed on ice while transported to the laboratory for analysis. Hb typing was determined in all blood samples by capillary electrophoresis. These measurements were carried out on CAPILLARYS™ 2, Sebia. All measurements were made in accordance with the manufacturer's instructions. Blood glucose and glycated hemoglobin were analyzed by the Dimension® RxL Max® clinical chemistry system (Siemens Healthcare Diagnostics Inc.). Multiple sequence alignment was carried out using MAFFT Multiple Sequence Alignment. The sequence of the camel Hb chain A (NCBI accession no P63106.1) was aligned with human Hb Chain A (NP_000508.1) and cow Hb chain A (NP_001070890.2). Similarly, the B chain of camel Hb (P68231.2) was aligned with human HbB chain (000509.1) and cow HbB chain (NP_001103977.1). The alignment output was color-coded according to ther identities.

Sequence homologies between the chains of Hb (camel versus human or cow) were calculated by using Blast-2-seq (http://blast.ncbi.nlm.nih.gov). The query and subject sequences were added in FASTA format and BLASTp was conducted.

3D structural similarity was investigated by superimposing camel Hb (PDB id 3GDJ) and human Hb (2W6V) using the PyMOL program (http://pymol.sourceforge.net).

The isoelectric points of A and B chains of camel, human and cow were computed using the ProtParam tool (http://web.expasy.org/protparam/).

pKa values and surface exposure of lysines were calculated for the A and B chains of camel and human using the PROPKA Web Interface (http://propka.ki.ku.dk/)

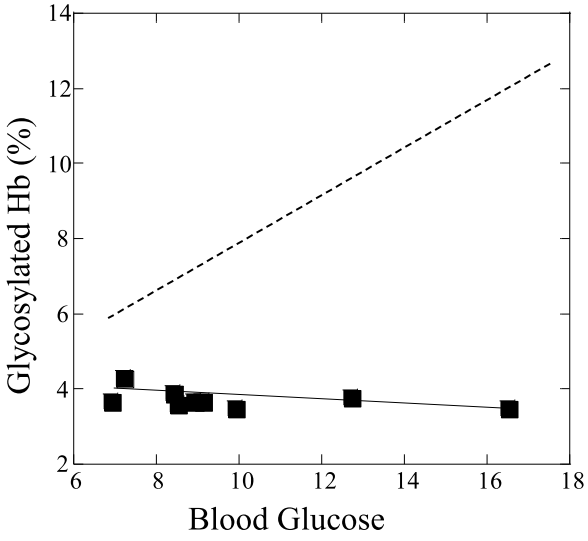


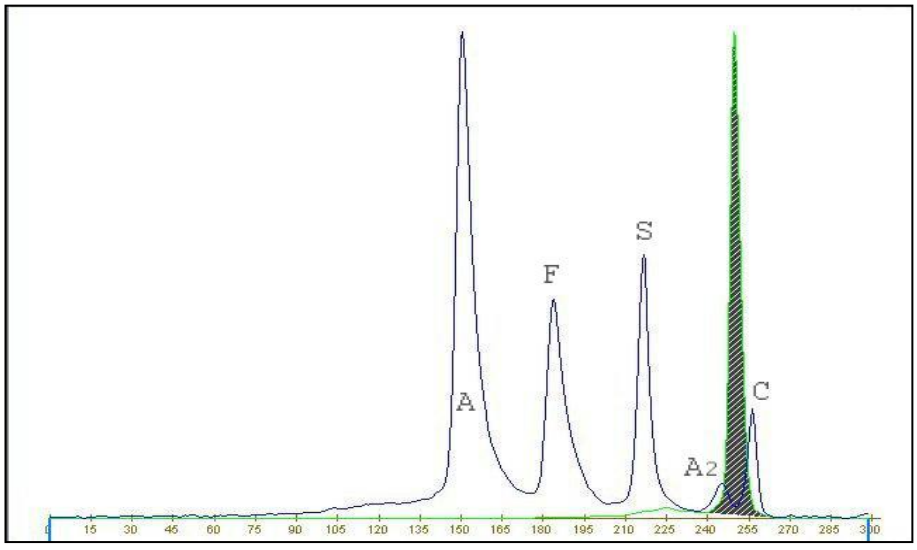
Fig. 1. Extent of glycation versus blood glucose
The figure shows actual data of camel blood samples (n=11). Camel blood glucose vs extent of glycation was determined. The square shows the actual data and the dashed line shows the calculated values for human, based on equation as described by (Nathan et al., 2008).

RESULTS AND DISCUSSION

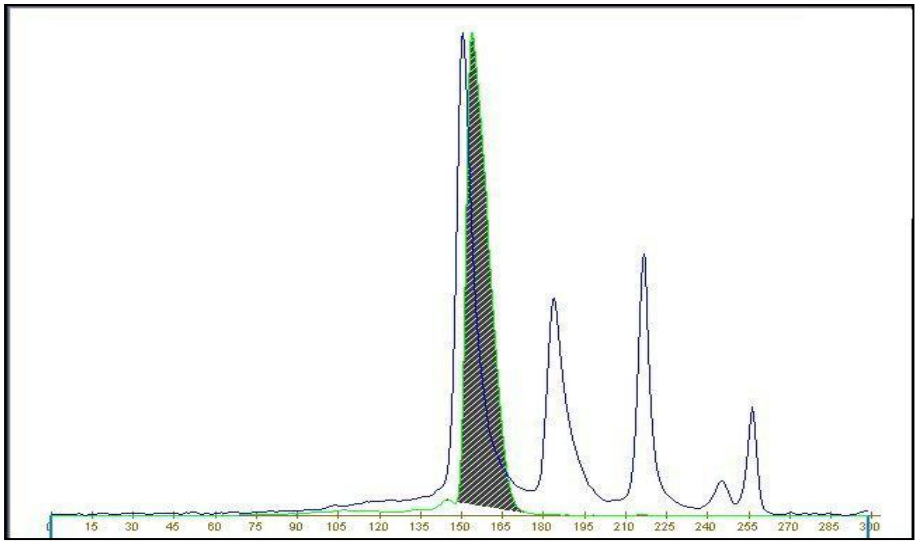
Table 1 shows blood glucose and glycated Hb (HbA1c) values for camel and cow. Camel showed higher blood glucose levels than cow (9.7 mM versus 5.7 mM), but a similar concentration of HbA1c (3.7% versus 3.2%). Even though the blood parameters in cattle are subjected to seasonal variations, the results were consistent with other studies, suggesting that camel had high blood glucose and a low level of Hb glycation (Abdalla et al., 1988; Al-Ali et al., 1988; al-Ali et al., 1990; Amin et al., 2007).

The glucose concentration in camel blood is high by human standard, and is observed only in unmanaged diabetic cases. However, the HbA1c in camel was lower than expected for a human with similar high blood glucose level. In humans, the HbA1c fraction is universally accepted as a long-term indicator

2A



2B

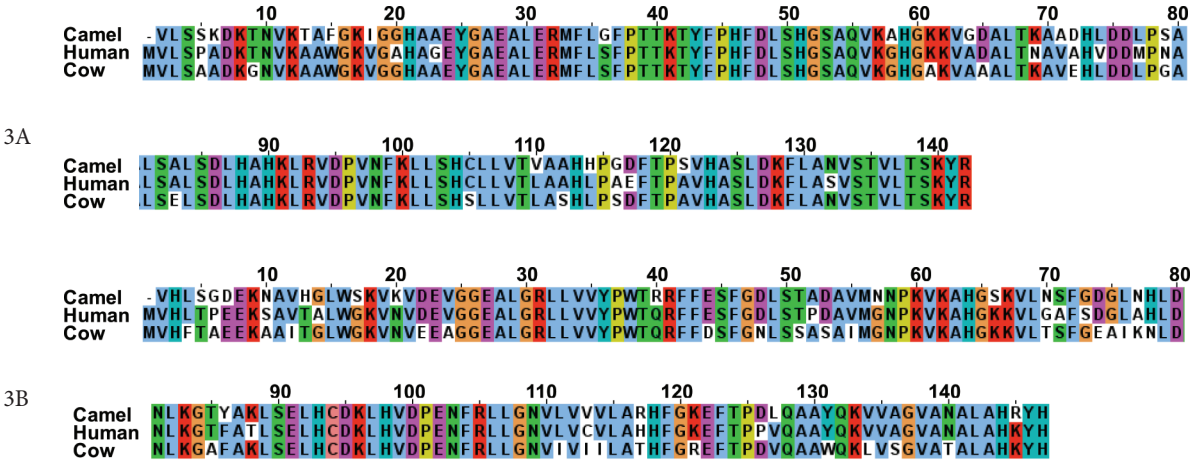


Figs. 2 A & B: Panel A- migratory position of camel Hb. Panel B shows migration of cow Hb under the same conditions. In both panels, the migratory positions of human Hb are shown for comparison.

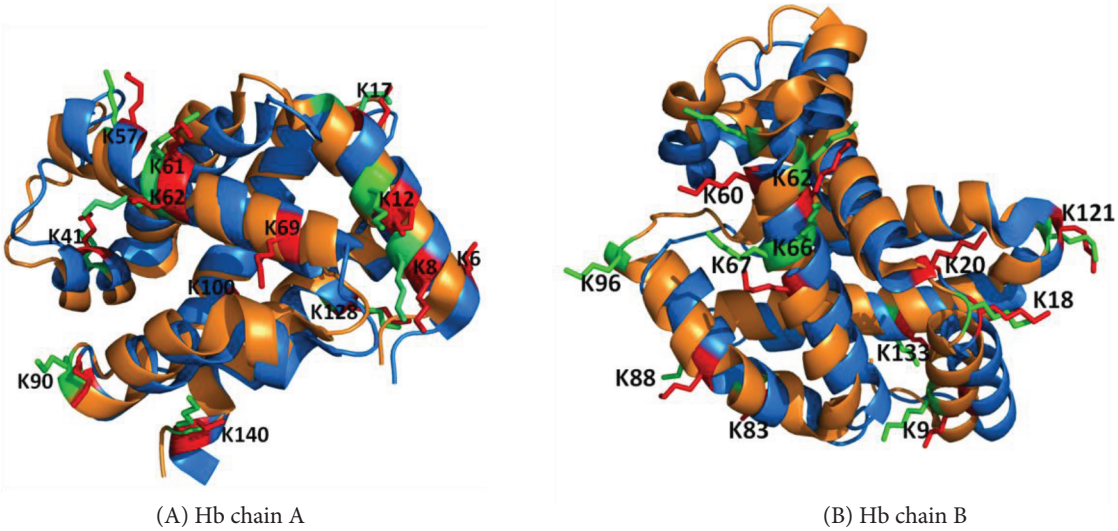
of average blood glucose concentration (Nathan et al., 2008; Rohlfing et al., 2002).

Glycation of hemoglobin has been reported to proceed via a non-enzymatic reaction; a reaction between amino groups of Hb and sugars leading to the formation of stable derivatives via the Amadori reaction (Bunn, 1981; Peacock, 1984). The linkage of sugar to the N-terminal valine residue of the β-chain of Hb gives rise to HbA1c, a type of Hb commonly associated with diabetes (Nathan et al., 1984; Rohlfing et al., 2002; Soranzo, 2011). The extent of HbA1c formation is directly related to the blood glucose levels in humans (Nathan et al., 2008), and possibly in other animals (Shahbazkia and Nazifi, 2008; Shahbazkia et al., 2010).

In this study, the observed range of glucose in camel blood was wide enough to permit the examination of a possible correlation between HbA1c and



Figs. 3. Multiple sequence alignment between camel, human and cow Hb.(A) Hb chain A (B) Hb chain B.



(A) Hb chain A (B) Hb chain B

Figs. 4. Camel Hb superimposed on human Hb using PyMol. Both figures emphasized on side chains of lysine residues (primary glycation sites) in the A and B chains of camel and human Hb. Side chain of lysine residues in camel and human Hb are shown in red and green, respectively.

blood glucose in the camel. Fig. 1 plots show the observed HbA1c versus blood glucose levels for all camel blood samples examined. The plot also shows the expected HbA1c values had the blood glucose values belonged to human subjects. This indicates that the Hb glycation in camel was drastically different from that of humans. The glycation of camel Hb showed a minimal to slightly negative correlation with blood glucose concentration, whereas that of human Hb is expected to be positively correlated with blood glucose.

A number of possible scenarios, physiological and/or chemical, could account for such results. For example, camel erythrocytes may restrict glucose transport and therefore camel hemoglobin might be exposed to lower glucose concentrations than suggested by the blood glucose. It is also possible that

Table 1. Blood glucose and glycated Hb values for camel and cow.

	Camel (n=11)		Cow (n=5)	
	Average ±SD	Observed Range	Average ±SD	Observed Range
Glucose (mM)	9.7 ± 2.9	6.9 -16.5	5.7 ± 0.7	4.8 - 6.5
HbA1c %	3.7 ± 0.23	3.5 - 4.3	3.2 ± 0.11	3.1 - 3.4

Table 2: Comparison of positively and negatively charged residues in the camel, human and cow Hb.

	Hemoglobin Chain A			Hemoglobin Chain B		
Residue	Camel	Human	Cow	Camel	Human	Cow
Lysine	13	11	11	11	11	12
Histidine	11	10	10	9	9	7
Arginine	3	3	3	6	3	4
Aspartic acid	10	8	8	9	7	5
Glutamic acid	3	4	5	7	8	9
pI[a]	9.02	8.72	8.07	8.06	6.74	8.71

[a]computed using ProtParam tool (http://web.expasy.org/protparam/).

the redox state of camel blood or the internal environment of erythrocytes as well as variations of blood pH may contribute to the reduction of HbA1c levels (Kurata et al., 1993). Other scenarios are also possible, but these factors, together with other post-translational modification, might be responsible for protecting camel Hb against glycation.

The mobility of camel Hb was examined by capillary electrophoresis (Fig. 2). Camel Hb consistently migrated at a position very close to that of human Hb C (Fig. 2A): camel Hb migrated 250±5 seconds in all samples examined (n=11). By contrast, cow Hb migrated to a position comparable to that of normal human HbA: all samples examined (n=5) migrated 155±5 seconds (Fig. 2B).

The biochemical basis for the migratory position of camel Hb, as well as the apparent resistance of camel Hb to glycation was investigated further via comparative analyses using bioinformatics tools: for sequence alignments (Fig. 3), charged amino acids compositions (Table 2), and 3D structural similarities (Fig. 4) between camel and human Hb. The difference in electrophoretic mobility between camel versus human Hb could be due to a difference in net electrical charges, shape, or exposure of amino acids. Protein glycation could be modulated by a number of glycation sites, their pKa values, and their surrounding environment and surface exposure.

The alignment of camel, cow and human hemoglobin chain A (Fig. 3A) and chain B (Fig. 3b) suggests that these chains are fairly conserved in these species, with more than 84% identity. A particularly interesting observation was that the amino acids around the positively charged amino groups are fairly conserved; it has been reported that such residues play an important role in the susceptibility of glycation (Shapiro et al., 1980; Baynes et al., 1989)

Camel Hb appeared to be more basic than either that of humans or cow (Table 2). It also contains more positively and negatively charged amino acids than either humans or cow. However, minor differences in the charges alone may not account for the

difference in electrophoretic mobility between camel and human Hb: the cow and human hemoglobin migrated nearly to the same position, despite the net differences in the number of charged amino acids as well as their calculated isoelectric points.

Fig. 4 superimposed 3D structures of camel and human Hb, with special emphasis on the lysine residues, the most probable sites of glycation. The two structures were virtually identical. All lysines of chain A and B of human versus the corresponding chains of camel Hb were very similar in pKa values and burial percentage; only one lysine of human chain A and two lysines of chain B had significant variations in pKa values (1 unit lower) than corresponding residues in camel Hb. These data indicated that the lysines of camel Hb might be more nucleophilic than those of human Hb, and thus more prone to glycation.

In summary, camel Hb appeared to be less susceptible to glycation than human Hb, and it also migrated to a position close to that of human HbC. An analysis of the number of charged residues, their pKa values, and surface exposure failed to provide an explanation for either of these observations.

Acknowledgments The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project no RGP – VPP – 151.

REFERENCES

Abdalla, O. M., Wasfi, I. A. and *F. A. Gadir* (1988). The Arabian race camel normal parameters--I. Haemogram, enzymes and minerals. *Comp. Biochem. Physiol. A: Physiol*. **90**, 237-239.

al-Ali, A. K., Husayni, H. A, and *D. M. Power* (1988). A comprehensive biochemical analysis of the blood of the camel (*Camelus dromedarius*). *Comp. Biochem. Physiol. B*. **89**, 35-37.

al-Ali, A. K., Rehaimi, A., Saba, R. and *D. M. Power* (1990). A comparative study of glycosylated haemoglobin level in the Arabian camel (*Camelus dromedarius*) during different seasons. *Comp. Biochem. Physiol. B*. **96**, 821-823.

al-Rehaimi, A. A., al-Ali, A. K., Mutairy, A. R. and *A. S. Dissanayake* (1989). A comparative study of enzyme profile of camel (*Camelus dromedarius*) hump and sheep (Ovis aries) tail tissues. *Comp. Biochem. Physiol. B* **93**, 857-858.

Ali, M. A., Nyberg, F., Chandranath, S. I. , Ponery, A. S., Adem, A. and *E. Adeghate* (2006). Effect of high-calorie diet on the prevalence of diabetes mellitus in the one-humped camel (*Camelus dromedarius*). *Ann. N. Y. Acad. Sci.*, **1084,** 402-410.

Amin, A. S., Abdoun, K. A. and *A. M. Abdelatif* (2007). Seasonal variation in blood constituents of one-humped camel (*Camelus dromedarius*). *Pak. J. Biol . Sci*, **10**, 1250-1256.

Barakat, M. Z. and *M. Abdel-Fattah* (1971). Seasonal and sexual variations of certain constituents of normal camel blood. *Zentralbl. Veterinarmed. A*. **18,** 174-178.

Baynes, J. W., Watkins, N. G., Fisher, C. I., Hull, C. J., Patrick, J. S., Ahmed, M. U., Dunn, J. A. and *S. R. Thorpe* (1989). The Amadori product on protein: structure and reactions. *Prog. Clin. Biol. Res.* **304**, 43-67.

Bogin, E. (2000). Clinical pathology of Camelides : present and future. *Revue. Méd. Vét*. **151**, 563-568.

Bunn, H. F. (1981). Nonenzymatic glycosylation of protein: Relevance to diabetes. *The Am. J. Med.***70**, 325-330.

Gaughan, J. B. (2011). Which physiological adaptation allows camels to tolerate high heat load – and what more can we learn?. *J. Camelid Sci.* **4**, 85-88.

Kataria, N., Kataria, A. K., Agarwal, V. K., Garg, S. L. and *M. S. Sahni* (2002). Effect of long-term dehydration on serum constituents in extreme climatic conditions in camel (*Camelus dromedarius*), *Indian J. Physiol. Pharmacol*. **46**, 218-222.

Kurata, M., Suzuki, M. and *N. S. Agar* (1993). Antioxidant systems and erythrocyte life-span in mammals. *Comp. Biochem. Physiol. B.* **106**, 477-487.

Mohamed, H. A. and *A. N. Hussein* (1999). Studies on normal haematological and serum biochemical values of the 'Hijin' racing camels (*Camelus dromedarius*) in Kuwait, *Vet. Res. Commun.* **23**, 241-248.

Nathan, D. M., Singer, D. E., Hurxthal, K. and *J. D. Goodson* (1984). The Clinical Information Value of the Glycosylated Hemoglobin Assay. *New Engl. J. Med.* **310**, 341-346.

Nathan, D. M., Kuenen, J., Borg, R., Zheng, H., Schoenfeld, D. and *R. J. Heine* (2008). Translating the A1C assay into estimated average glucose values. *Diabetes Care*. **31**, 1473-1478.

Nazifi, S., Rezakhani, A. and *H. R. Gheisari* (1998). Physical, biochemical and cytologic properties of blood and synovial fluid in clinically normal adult camel (Camelus dromedarius). *Zentralbl. Veterinarmed A*. **45**, 155-160.

Ouajd, S. and *B. Kamel* (2009). Physiological Particularities of Dromedary (Camelus dromedarius) and Experimental Implications, *Scand. J. Lab. Anim. Sci*, **36**, 19-29.

Peacock, I. (1984). Glycosylated haemoglobin: measurement and clinical use. *J. Clin. Pathol*. **37**, 841-851.

Rohlfing, C. L., Wiedmeyer, H. M., Little, R. R., England, J. D., Tennill, A. and *D. E. Goldstein* (2002). Defining the relationship between plasma glucose and HbA(1c): analysis of glucose profiles and HbA(1c) in the Diabetes Control and Complications Trial. *Diabetes Care*, **25**, 275-278.

Shahbazkia, H. and *S. Nazifi* (2008). Glycated haemoglobin (HbG) as a stable indicator of blood glucose status in ostrich, *Comp. Clin. Pathology.* **17**, 9-12.

Shahbazkia, H. R., Shareghi, B. and *M. Aminlari* (2010). Glycated Hemoglobin is an Indicator of Blood Glucose Status in Horses: Preliminary Study. *J. Equine Vet. Sci.* **30**, 47-49.

Shapiro, R., McManus, M. J., Zalut, C. and *H. F. Bunn* (1980). Sites of nonenzymatic glycosylation of human hemoglobin A. *J. Biol. Chem*. **255**, 3120-3127.

Soranzo, N. (2011). Genetic Determinants of Variability in Glycated Hemoglobin (HbA_{1c}) in Humans: Review of Recent Progress and Prospects for Use in Diabetes Care, *Curr. Diab. Rep.* **11**, 562-569.

Yagil, R. and *G. M. Berlyne* (1977). Glucose loading and dehydration in the camel, *J. Appl. Physiol*. **42**, 690-693.