

Fetal hemoglobin levels are related to metabolic control in diabetic subjects

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Abstract

We have investigated the relationship between fetal hemoglobin (HbF) levels and metabolic control in subjects with insulin-dependent (N = 79) and non-insulin-dependent diabetes mellitus (N = 242). HbF and hemoglobin A1c (HbA1c) levels were increased in subjects with type 1 and type 2 diabetes as compared to levels in nondiabetic individuals ($P < 0.0001$), and were significantly higher in type 1 than in type 2 diabetes subjects. Lower levels of HbA1c and HbF were observed in type 2 diabetes subjects treated by diet, intermediate levels in those treated with oral hypoglycemic agents, and higher levels in those treated with insulin. HbF and HbA1c levels were correlated in type 1 diabetes ($R^2 = 0.57$, $P < 0.0001$) and type 2 diabetes ($R^2 = 0.58$, $P < 0.0001$) subjects. Following intense treatment, twelve diabetic patients showed significant improvement both in HbA1c and HbF values. We conclude that increased HbF levels reflect poor metabolic control in subjects with diabetes mellitus.

Key words

- Fetal hemoglobin
- HbA1c
- Glycated hemoglobin
- Diabetes mellitus
- Type 1 diabetes
- Type 2 diabetes

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Introduction

Several hemoglobin subfractions are formed by glycation of hemoglobin A (HbA), including HbA1a, HbA1b, the labile intermediate form (Hb-L-A1c) and glycated hemoglobin A1c (HbA1c). HbA1c is formed in erythrocytes by a post-translational non-enzymatic two-step reaction. Glucose is first linked to the N-terminal valine residue of the beta chain of HbA forming Hb-L-A1c, which subsequently undergoes an Amadori rearrangement to yield the stable form. HbA1c levels reflect the average blood glucose con-

centration in the preceding 2 to 3 months (1,2), and the measurement of HbA1c has become an important reference value in monitoring the treatment of diabetes (3,4).

Fetal hemoglobin (HbF) is produced in a subpopulation of erythrocytes termed "F-cells" and is the predominant hemoglobin in fetal life and early infancy. During the first year of life, HbF is gradually replaced by hemoglobin A, and only small amounts of HbF (<1.0%) can be found in adult life (5). HbF levels were shown to be increased in some subjects with type 1 diabetes (6-8), but data on HbF levels in type 1 diabetes are

scarce. Moreover, no data are available for subjects with type 2 diabetes, and the correlation of HbF levels with the quality of metabolic control in diabetic subjects remains unclear. In the present study, we have investigated the relation between HbF and metabolic control in subjects with type 1 and type 2 diabetes.

Material and Methods

Subjects

HbA1c and HbF levels were measured in 79 subjects with type 1 diabetes and 242 subjects with type 2 diabetes. All subjects were outpatients in the diabetes clinics of the Santa Casa or the Felício Rocho Hospitals in the city of Belo Horizonte, Brazil. Diabetes mellitus was defined according to World Health Organization criteria (9). Ascertainment of type 1 diabetes was based on a diagnosis of diabetes before the age of 30 years, the presence of ketoacidosis or weight loss at diagnosis, insulin-dependence thereafter, and body mass index (BMI) <25.0 kg/m². Ascertainment of type 2 diabetes was based on the diagnosis of diabetes after the age of 40 years, with no signs of ketoacidosis. Insulin-treated type 2 diabetes subjects with a BMI <28.0 kg/m² and subjects with

diabetes diagnosed between 30 and 40 years were excluded to avoid misdiagnosis of type 1 diabetes. Individuals younger than 10 years and those presenting nephropathy were also excluded from the study. Two groups of healthy subjects matched by age with the type 1 and type 2 diabetes patients were used as controls. Demographic and clinical data of patients and controls are shown in Table 1. The protocol was approved by the Santa Casa Ethics Committee. Subjects gave informed consent to participate in the study.

HbA1c and HbF were also measured in 12 diabetic subjects (3 type 1 and 9 type 2 diabetes) before and after 6 to 16 weeks (average 12 weeks) of intensive treatment, to test the effects of a better metabolic control on HbF levels. Frequent blood glucose testing and insulin dose adjustments were scheduled for the type 1 diabetes patients, while 10 units/day of insulin were added to the treatment of type 2 diabetes patients.

HbA1c and HbF assay

HbA1c and HbF were measured in whole blood plus EDTA with a high-performance liquid chromatography (HPLC) ion-exchange analyzer (Glycated Hemoglobin Analyser, Merck Hitachi L-9100, Tokyo, Japan). This

Table 1 - Demographic and biological profile of patients and controls.

Data are reported as means \pm SD. OHA, Oral hypoglycemic agents. ^aP<0.05 compared to control group 1; ^bP<0.05 compared to control group 2 (ANOVA); ^cP<0.05 compared to type 2 diabetes subjects (ANOVA followed by the Tukey-Kramer HSD test).

	Type 1 diabetes	Control group 1	Type 2 diabetes	Control group 2	P
Subjects (N)	78	129	242	81	
Sex (M/F)	34/44	42/87	107/135	21/60	-
Age (years)	25 \pm 12	25 \pm 7	60 \pm 12	58 \pm 9	-
Age at diagnosis (years)	12 \pm 7	-	51 \pm 11	-	-
Treatment: diet/OHA/insulin (%)	0/0/100	-	27/48/25	-	-
Fasting glucose (mM)	10.1 \pm 4.9 ^a	4.6 \pm 0.4	10.0 \pm 3.6 ^b	5.0 \pm 0.6	<0.0001
HbA1c (%)	7.9 \pm 2.1 ^{ac}	3.5 \pm 0.4	7.1 \pm 2.0 ^b	3.8 \pm 0.4	<0.0001
HbF (%)	0.99 \pm 0.48 ^{ac}	0.51 \pm 0.34	0.82 \pm 0.35 ^b	0.42 \pm 0.16	<0.0001

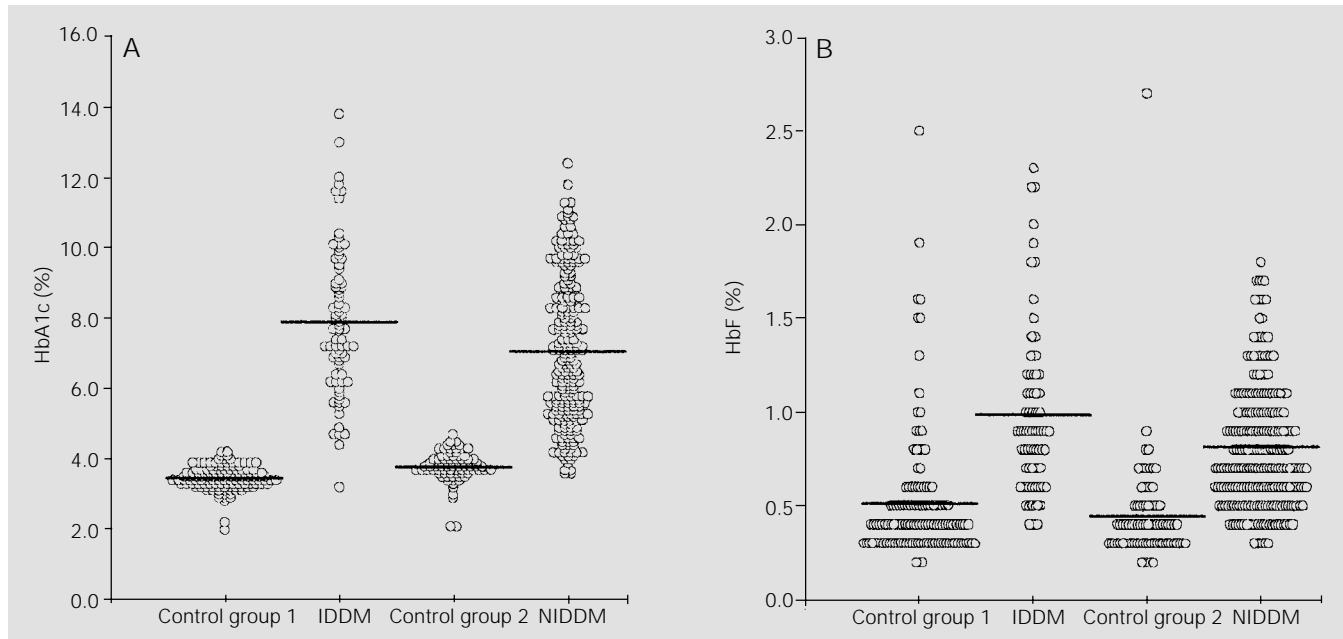


Figure 1 - HbA1c (A) and HbF (B) levels in IDDM (type 1 diabetes) and NIDDM (type 2 diabetes) subjects and in their respective control groups. Solid lines denote means.

is a fully automated analyzer consisting of an auto-sampler that withdraws the sample from the rack for dilution and hemolysis. The hemolyte is then pumped into an analytic separating column (CCMpack HB-S) warmed to 40°C by a column oven for high-speed analysis. The fractions (A1a, A1b, F, L-A1c, A1c and A0 hemoglobin) eluted from the column are detected with a dual wavelength photometer and processed by a built-in microcomputer to identify and calculate the area of the peaks. The intra-assay and inter-assay coefficients of variation (CV) for HbF were 7.9 and 7.7%, respectively. The intra-assay CV for HbA1c was less than 2.0%, and the interassay CV was 3.8 or 5.6% for normal reference values or high values of HbA1c, respectively.

HbF levels were also measured in 17 subjects (8 type 2 diabetes and 9 control individuals) by the alkali denaturation method (10), which is considered to be the most sensitive method to detect low HbF levels. We found a strong correlation between the results obtained by HPLC and those ob-

tained by the alkali denaturation method ($R^2 = 0.90$; $P < 0.0001$).

Statistics

Data are reported as means \pm SD. Differences between groups were assessed by the paired Student *t*-test and by analysis of variance (ANOVA) followed by the Tukey-Kramer HSD (honestly significant difference) post-test for multiple comparisons (11). Linear regression analyses were performed to determine the associations of clinical and biological parameters. Data were analyzed with the SPSS for Windows 7.0 software (SPSS Inc., Chicago, IL).

Results

HbA1c levels were increased in subjects with type 1 and type 2 diabetes as compared to subjects from control groups 1 and 2 (Table 1, Figure 1), and were significantly higher in type 1 than in type 2 diabetes subjects. HbF levels were increased in both

groups of diabetic patients as compared to the respective controls. HbF levels were also significantly higher in type 1 than in type 2 diabetes subjects. No sex-related differences were observed in HbF or HbA1c levels in any of the four groups (data not shown).

HbA1c levels in type 2 diabetes subjects treated with diet, oral hypoglycemic agents (OHA) and insulin were 5.8 ± 1.7 , 7.2 ± 1.9 and $8.2 \pm 1.9\%$ ($P < 0.0001$; all Tukey-Kramer HSD comparisons between pairs $P < 0.05$), respectively. HbF levels in these groups were 0.62 ± 0.24 , 0.83 ± 0.33 and $1.0 \pm 0.37\%$ ($P < 0.0001$; all Tukey-Kramer HSD comparisons between pairs $P < 0.05$), respectively.

HbF values were correlated to HbA1c values in type 1 ($R^2 = 0.57$, $P < 0.0001$) and type 2 diabetes ($R^2 = 0.58$, $P < 0.0001$) subjects but not in control subjects (Figure 2). HbA1c values were correlated with fasting plasma glucose values in diabetic subjects (pooled data $R^2 = 0.29$, $P < 0.0001$) and mildly correlated in control subjects (pooled data $R^2 = 0.04$, $P < 0.009$). HbF values were mildly

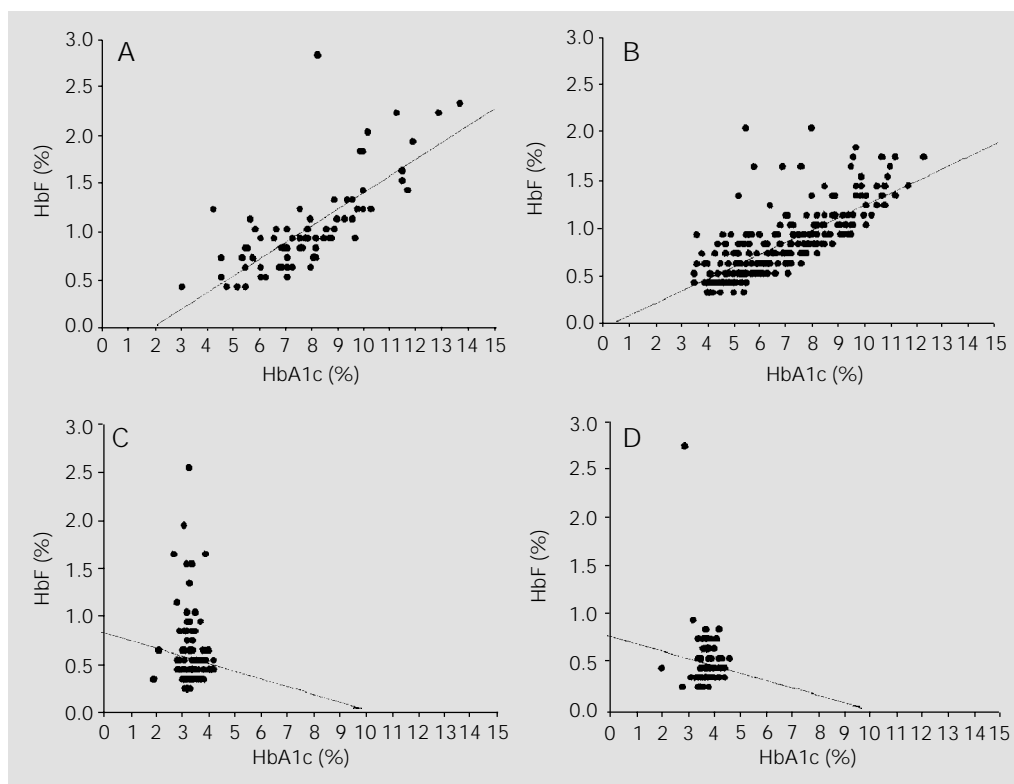
correlated with fasting plasma glucose values only in diabetic subjects (pooled data $R^2 = 0.09$, $P < 0.0001$). HbA1c and HbF values were correlated with the duration of diabetes in type 2 diabetes but not in type 1 diabetes subjects (data not shown).

Figure 3 shows HbA1c and HbF values in the 12 diabetic subjects who received intensive treatment. Basal and post-treatment HbA1c levels were 7.9 ± 1.6 and $5.9 \pm 1.2\%$ ($P < 0.0001$), respectively. Concomitant basal and post-treatment HbF levels were 0.83 ± 0.22 and $0.58 \pm 0.16\%$ ($P < 0.0001$), respectively. Average improvement with treatment was 24.7% for HbA1c values and 29.7% for HbF values (Figure 3). Improvements in these parameters were highly correlated ($R^2 = 0.76$; $P < 0.0001$).

Discussion

We have observed increased levels of HbF in subjects with type 1 and type 2 diabetes. HbF levels were positively corre-

Figure 2 - Correlations between HbA1c and HbF levels in (A) type 1 diabetes subjects ($R^2 = 0.57$, $P < 0.0001$), (B) type 2 diabetes subjects ($R^2 = 0.58$, $P < 0.0001$), (C) control group 1 ($R^2 = 0.007$, $P = 0.36$), and (D) control group 2 ($R^2 = 0.012$, $P = 0.33$).



lated with HbA1c levels in diabetic but not in nondiabetic individuals. Subjects with type 1 diabetes had higher levels of both HbA1c and HbF than the subjects with type 2 diabetes. Furthermore, we have observed lower levels of both HbA1c and HbF in type 2 diabetes subjects who were treated with diet only, intermediate levels in those treated with OHA, and higher levels in the subjects treated with insulin. It is noteworthy that in type 1 diabetes subjects the prevalence of high HbF levels (see below) starts to increase gradually at an HbA1c level of 7.7% and at an HbA1c level of 9.9%, all diabetic patients presented high HbF levels (data not shown). In type 2 diabetes subjects, the prevalence of high HbF levels starts to increase at HbA1c levels of 7.0% and at an HbA1c level of 10.2% all patients presented high HbF levels. Since it is well established that HbA1c levels reflect the quality of metabolic control in diabetic subjects, all these data suggest that high HbF levels might be associated with poor metabolic control. Moreover, we have observed that a decrease in HbA1c levels, and thus a better metabolic control, induced by intensive treatment in type 1 and type 2 diabetes subjects is associated with a decrease in HbF levels.

An increased prevalence of high HbF levels ranging from 13 to 38% of cases has been reported in type 1 diabetes subjects (6-8). Our data are in agreement with these reports. We have observed that 32% of the type 1 diabetes subjects of our cohort presented HbF levels higher than 1.0% (Figure 1), which is the level corresponding to the 90th percentile of HbF distribution in the control groups. Furthermore, 23% of the type 2 diabetes subjects in our cohort presented HbF levels higher than 1.0%. To our knowledge, this is the first demonstration of increased HbF levels in type 2 diabetes patients. Note that 99% of the subjects with type 1 diabetes (all but one) and 97% of the subjects with type 2 diabetes (all but seven) presented HbA1c levels higher than 4.1%

(Figure 1), which is the level corresponding to the 90th percentile of HbA1c distribution in the control groups. These data suggest that, although HbF and HbA1c are well correlated in diabetic subjects, HbA1c is more accurate as a marker of diabetes.

The mechanisms leading to high HbF levels in diabetes are unknown. It has been suggested that high HbF levels could be related to a direct effect of insulin therapy (7,12). However, our data do not agree with this hypothesis. Indeed, we have observed

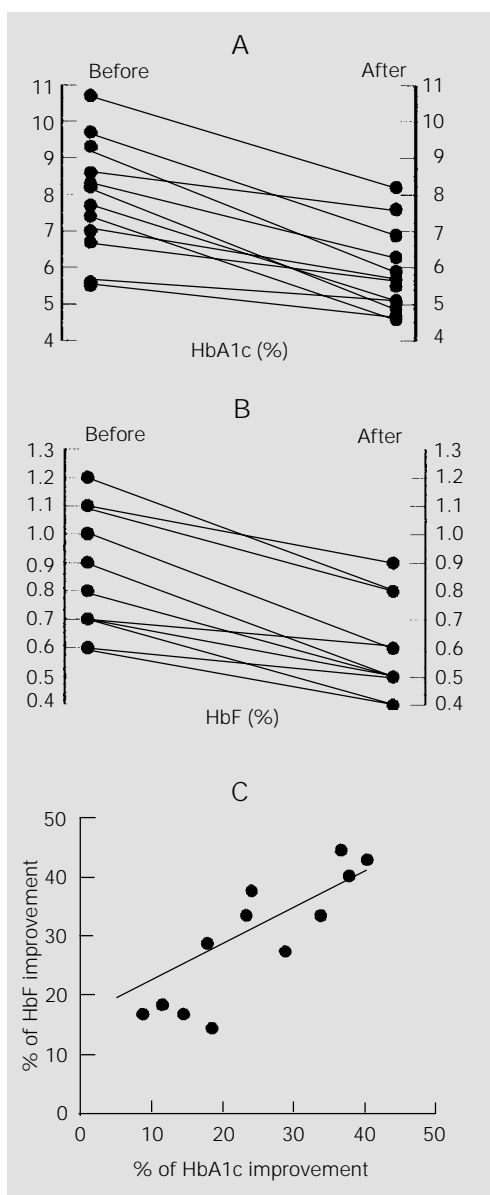


Figure 3 - HbA1c (A) and HbF (B) levels before and after intensive treatment. (C) Correlation between the percentage of improvement in HbA1c and HbF levels ($R^2 = 0.76$, $P < 0.0001$).

that type 2 diabetes subjects with poorly controlled diabetes (high HbA1c levels) treated with diet or OHA also present high HbF levels which are positively correlated with HbA1c levels (data not shown). It is possible that HbF synthesis might be increased by stimuli resulting from poor metabolic control. Many studies have demonstrated that the expression of the γ -globin gene can be pharmacologically induced by short-chain fatty acids, especially those with 2 to 5 carbons like acetate and butyric acid (13-15). It has been speculated that products of ketoacidosis present in poorly controlled type 1 diabetes subjects could induce γ -globin gene expression, thus increasing HbF levels. Although type 2 diabetes patients are keto-sis-resistant (16), several reports have shown that type 2 diabetes patients with poor metabolic control present increased circulating levels of short-chain fatty acids (17-21). Clearly, these mechanisms must be further investigated.

The observation of increased HbF levels in diabetes has practical consequences for the assessment of HbA1c in diabetic pa-

tients. Since HbF migrates closer to HbA1c in many electrophoresis and ion-exchange chromatography systems (22,23), falsely increased levels of HbA1c may be detected if the technique does not separate the two hemoglobin fractions (24). In this study 34 diabetic patients presented high HbF levels (>1.0%) with normal L-A1c (>1.0%), which implies that there was no contamination between these Hb fractions in the chromatographic analysis (data not shown).

In conclusion, we observed high HbF levels in type 1 and type 2 diabetes subjects. HbF levels were highly correlated with HbA1c levels in diabetic but not in non-diabetic individuals. Increased HbF levels in diabetic subjects seem to reflect poor metabolic control. However, the mechanisms resulting in high HbF levels in diabetes mellitus remain poorly understood.

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References

- Goldstein DE, Little RR, Wiedmeyer H, England JD & Mackenzie EM (1986). Glycated hemoglobin: methodologies and clinical applications. *Clinical Chemistry*, 32 (Suppl 1): B64-B70.
- Cefalu WT, Wang ZQ, Bell-Farrow A, Kiger FD & Izlar C (1994). Glycohemoglobin measured by automated affinity HPLC correlates with both short-term and long-term antecedent glycemia. *Clinical Chemistry*, 40: 1317-1321.
- Jovanovic L & Peterson CM (1981). The clinical utility of glycosylated hemoglobin. *American Journal of Medicine*, 70: 331-338.
- Larsen ML, Horder M & Mogensen EF (1990). Effect of long-term monitoring of glycosylated hemoglobin levels in insulin-dependent diabetes mellitus. *New England Journal of Medicine*, 323: 1021-1025.
- Rochette J, Craig JE & Thein SL (1994). Fetal hemoglobin levels in adults. *Blood Reviews*, 8: 213-224.
- Mullis P, Schuler JJ & Zuppinger K (1989). Increased prevalence of fetal haemoglobin in type 1 (insulin-dependent) diabetes mellitus. *Diabetologia*, 32: 227-230.
- Koskinen LK, Latela JT & Koivula TA (1994). Fetal hemoglobin in diabetic patients. *Diabetes Care*, 17: 828-831.
- Diem P, Mullis P, Hirt A, Schuler JJ, Burgi W, Zuppinger K & Teuscher A (1993). Fetal haemoglobin levels in adult type 1 (insulin-dependent) diabetic patients. *Diabetologia*, 36: 129-132.
- World Health Organisation (1985). Diabetes mellitus. Report of a WHO Study Group. Technical Report Series, WHO, Geneva, No. 727.
- Betke K, Marti HR & Schlicht I (1959). Estimation of small percentages of fetal haemoglobin. *Nature*, 184: 1877-1878.
- Kramer CY (1956). Extension of multiple range tests to group means with unequal numbers of replications. *Biometrics*, 12: 309-310.
- Kilpatrick ES, Small M, Rumley A & Dominiczak MH (1993). Increased fetal hemoglobin in insulin-treated diabetes mellitus contributes to the imprecision of glycohemoglobin measurements. *Clinical Chemistry*, 39: 833-835.
- Liakopoulou E, Blau CA, Li Q, Josephson B, Wolf JA, Fournarakis B, Aisys V, Dover G, Papayannopoulou T & Stamatoyannopoulos G (1995). Stimulation of fetal hemoglobin production by short chain fatty acids. *Blood*, 86: 3227-3235.
- Constantoulakis P, Papayannopoulou T & Stamatoyannopoulos G (1988). Alpha-amino-N-butyric acid stimulates fetal hemoglobin in the adult. *Blood*, 72: 1961-1967.
- Stamatoyannopoulos G, Blau CA, Nakamoto B, Josephson B, Li Q, Liakopoulou E, Pace B, Papayannopoulou T, Brusilow SW & Dover G (1994). Fetal hemoglobin induction by acetate, a product of butyrate catabolism. *Blood*, 84:

- 3198-3204.
16. Nosadini R, Avogaro A, Doria A, Fioretto F, Trevisan R & Morocutti A (1989). Ketone body metabolism: a physiological and clinical overview. *Diabetes/Metabolism Reviews*, 5: 299-319.
 17. Papadakis M & Grunfeld G (1986). Ketonuria in hospitalized patients with non-insulin-dependent diabetes mellitus. *Diabetes Care*, 9: 596-600.
 18. Cook GA (1987). The hypoglycemic sulfonylurea glyburide and tolbutamide inhibit fatty acid oxidation by inhibiting carnitine palmitoyl-transferase. *Journal of Biological Chemistry*, 262: 4968-4972.
 19. Singh BM, Krentz AJ & Natrass M (1993). Insulin resistance in the regulation of lipolysis and ketone body metabolism in non-insulin dependent diabetes is apparent at very low insulin concentrations. *Diabetes Research and Clinical Practice*, 20: 55-62.
 20. Avogaro A, Valerio A, Gnudi L, Maran A, Zolli M, Duner E, Riccio A, Del Prato S, Tiengo A & Nosadini R (1992). Ketone body metabolism in NIDDM. Effect of sulfonylurea treatment. *Diabetes*, 41: 968-974.
 21. Avogaro A, Crepaldi C, Miola M, Maran A, Pengo V, Tiengo A & Del Prato S (1996). High blood ketone body concentration in type 2 non-insulin dependent diabetic patients. *Journal of Endocrinological Investigation*, 19: 99-105.
 22. Gruber CA & Krets MD (1979). Quantitation of hemoglobin A1a+b and hemoglobin A1c by automated "high performance" liquid chromatography. *Clinical Chemistry*, 25: 1970-1971.
 23. Bulusu S (1995). Variant haemoglobin may affect measurements. *British Medical Journal*, 310: 749-751.
 24. Goldstein DE & Little RR (1994). More than you ever wanted to know (but need to know) about glycohemoglobin testing (Editorial). *Diabetes Care*, 17: 938-939.