Increased body fat mass reduces the association between fructosamine and glycated hemoglobin in obese type 2 diabetes patients

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ABSTRACT
Obesity is increasing in patients with type 2 diabetes. A possible reduced association between fructosamine and glycated hemoglobin (HbA1c) in obese individuals has been previously discussed, but this has never been specifically evaluated in type 2 diabetes, and the potential influence of body fat mass and fat distribution has never been studied. We studied 112 type 2 diabetes patients with assessment of fat mass, liver fat and fat distribution. Patients with body mass index (BMI) above the median (34.9 kg/m²), versus BMI below the median, had a correlation coefficient between fructosamine and HbA1c significantly reduced (r = 0.358 vs r = 0.765). In the whole population, fructosamine was correlated negatively with BMI and fat mass. In multivariate analysis, fructosamine was associated with HbA1c (positively) and fat mass (negatively), but not with BMI, liver fat or fat distribution. The association between fructosamine and HbA1c is significantly reduced in the most obese type 2 diabetes patients, and this is mostly driven by increased fat mass.

INTRODUCTION
Because protein glycation is involved in diabetes complications, the clinical use of glycated hemoglobin (HbA1c), evaluating protein glycation during a 3-month period, is regarded as a gold standard for measurement of glycemic control. Fructosamine, a marker of plasma protein glycation, has also been shown to be of great value in determining overall glyceric control during a 2–3-week period. Measurement of fructosamine is particularly useful in situations in which HbA1c measurement is not reliable, in patients with rapid changes of glucose homeostasis and for identifying hyperglycemia before noticeable changes in HbA1c might occur. Fructosamine correlates rather well with HbA1c, with correlation coefficients ranging from 0.55 to 0.80.

However, the association between HbA1c and fructosamine is not clear in type 2 diabetes patients with obesity. Two studies carried out with first-generation fructosamine assays reported a reduced association between fructosamine and HbA1c in obese individuals compared with lean individuals, whereas another did not show any influence of body mass index (BMI) on fructosamine. However, these studies did not specifically study obese type 2 diabetes patients, and were not carried out with the more recent fructosamine assays. Furthermore, we do not know whether the association between HbA1c and fructosamine is influenced only by total bodyweight or more specifically by body fat mass, and potentially by liver fat, visceral fat or subcutaneous fat. Because obesity is becoming frequent among type 2 diabetes patients, it is important to clarify this point.

This prompted us to carry out a prospective study in a population of type 2 diabetes patients with a large BMI range including measurements of body fat mass, liver fat, visceral fat and subcutaneous fat.

METHODS
This prospective single-center study was approved by our regional ethics committee, and written informed consent was
obtained from all patients before study inclusion (trial registered as NCT02085876).

We included 112 patients with type 2 diabetes referred to our center for poorly controlled diabetes (HbA1c >7%). Exclusion criteria were severe hepatic impairment (aspartate aminotransferase or alanine aminotransferase >3-fold the upper limit of normal), hyper- or hypothyroidism, macroproteinuria, or renal function impairment (creatinine clearance <30 mL/min). Patients with alcohol and/or drug abuse, treatment with antidiabetic agents that might modify body fat composition and liver fat content (thiazolidinediones, glucagon-like peptide-1 agonists or sodium–glucose cotransporter 2 inhibitors) were not included.

All patients included in the study had a physical examination and fasting blood sampling for biological measurements.

Body fat mass and fat-free mass were assessed for each patient by dual-energy X-ray, which is considered as the reference method, with triplicate measurements, as recommended.

Liver fat content of the patients was obtained using a 3.0 Tesla Magnetom TRIO TIM whole body system (Siemens, Erlangen, Germany), as previously described. Visceral and subcutaneous fat areas were assessed in each patient by Magnetic Resonance Imaging at the level of the L4/L5 intervertebral disc.

Glycemia, total cholesterol, high-density lipoprotein cholesterol and triglycerides were quantitated on a Vista analyzer with dedicated reagents (Siemens Healthcare Diagnostics, Deerfield, IL, USA). Low-density lipoprotein cholesterol was calculated using the Friedewald formula, as serum triglyceride levels were <3.8 mmol/L. HbA1c was measured by high pressure liquid chromatography with a Tosoh G8 analyzer (Tosoh Bioscience, Tokyo, Japan). The Chronic Kidney Disease Epidemiology Collaboration equation was used to calculate the glomerular filtration rate. Fructosamine was measured in plasma using a colorimetric assay with nitrotetrazolium blue (Horiba, Montpellier, France) on a Dimension Vista Lab system (Siemens Healthcare Diagnostics).

The glycation gap (G-Gap) was calculated for each patient as the difference between measured HbA1c and HbA1c predicted from the fructosamine level. Fructosamine-predicted HbA1c was calculated as ((fructosamine-mean fructosamine) / standard deviation of fructosamine) × standard deviation of HbA1c + mean HbA1c according to the Macdonald method. A positive G-Gap would denote the fructosamine predicted HbA1c underestimating the true HbA1c.

Data are reported as mean ± standard deviation. We used the SPSS software package (SPSS, Chicago, IL, USA) to carry out the statistical analysis. Student’s t-test was used to compare the means between two groups. Linear regression analysis was used to determine the Pearson correlation coefficients (r). Stepwise multivariate linear regression was used to carry out multivariate analyses. Statistical significance was considered for P-values <0.05.

**RESULTS**

**Characteristics of the patients**

The clinical and biological characteristics of the patients with type 2 diabetes are shown in Table 1. Among the 112 type 2 diabetes patients, 101 were taking metformin, 49 were taking sulfonylureas, 33 were taking insulin, 26 were taking dipeptidyl peptidase-4 inhibitors, 22 were taking glinides and one was taking acarbose. The median BMI of the entire population was 34.9 kg/m². The patients were then divided into two groups according to BMI, above or below the median (Table 1). Patients with BMI above the median showed significantly higher fat mass, liver fat content, visceral fat and subcutaneous fat than those with BMI below the median (Table 1). Interestingly, the G-Gap was significantly higher in patients with BMI above the median (vs below the median) and positive, indicating underestimation of the true HbA1c with fructosamine (Figure 1b). The proportion of patients taking antidiabetic drugs affecting postprandial glucose was not different between the two groups: glinides (12 vs 10, P = 0.60), and acarbose (1 vs 0, P = 0.31).

**Association between HbA1c and fructosamine**

For the entire population, fructosamine was highly correlated with HbA1c (r = 0.532, P < 0.0001). However, the correlation was totally different when the analysis was carried out in each group separately. Indeed, the correlation between fructosamine and HbA1c was strong in the patients with BMI below the median (r = 0.765, P < 0.0001), whereas it was much weaker in the patients with BMI above the median (r = 0.358, P = 0.007). As shown in Figure 1a, the slope of the regression line between fructosamine and HbA1c was significantly steeper in patients with BMI below the median (β = 33.695x + 106.19) than in patients with BMI above the median (β = 11.489x + 294.7), and the slopes of the two regression lines were significantly different (P = 0.002). Fasting blood glucose was correlated with HbA1c to a similar extent in both groups, and the correlation coefficients were not statistically different between the two groups. HbA1c was not correlated with BMI in the whole diabetes population (r = −0.06, P = 0.53), as in each subgroup of patients with BMI below and above the median.

**Factors associated with fructosamine**

In the entire population, fructosamine was correlated positively with HbA1c (r = 0.532, P < 0.0001) and fasting blood glucose (r = 0.366, P < 0.0001), and negatively with BMI (r = −0.252, P = 0.008) and fat mass (r = −0.296, P = 0.002). No significant correlations were found between fructosamine, on the one hand, and free-fat mass (r = 0.085, P = 0.38), liver fat (r = −0.147, P = 0.12), subcutaneous fat (r = −0.164, P = 0.09) or visceral fat (r = −0.02, P = 0.84), on the other hand. Fructosamine was not significantly correlated with age, diabetes duration, hematocrit, albumin or protein.
Table 1 | Clinical and biological characteristics of the 112 type 2 diabetes patients

|                          | All patients (n = 112) | Patients with BMI below the median (n = 56) | Patients with BMI above the median (n = 56) | P-value (below median BMI vs above median BMI) |
|--------------------------|------------------------|--------------------------------------------|--------------------------------------------|-----------------------------------------------|
| Sex ratio (male/female)  | 65/47                  | 36/20                                      | 29/27                                      | NS (P = 0.15)                                |
| Age (years)              | 57.5 ± 11.5            | 59.1 ± 11.9                                | 55.8 ± 11.0                                | NS (P = 0.13)                                |
| Diabetes duration (years)| 10.1 ± 8.7             | 126 ± 9.6                                  | 76 ± 7.1                                   | <0.002                                        |
| Bodyweight (kg)          | 100.8 ± 19.7           | 87.4 ± 12.6                                | 113.4 ± 17.2                               | <0.0001                                       |
| BMI (kg/m²)              | 35.9 ± 6.7             | 30.6 ± 2.8                                 | 41.1 ± 5.2                                 | <0.0001                                       |
| HbA1c (%)                | 9.8 ± 2.1              | 9.8 ± 1.9                                  | 9.9 ± 2.3                                  | NS (P = 0.62)                                |
| Fructosamine (µmol/L)    | 422 ± 80               | 433 ± 84                                   | 409 ± 77                                   | NS (P = 0.11)                                |
| Fasting blood glucose (mmol/L) | 9.85 ± 3.40  | 9.95 ± 3.69                                | 9.75 ± 3.12                                | NS (P = 0.75)                                |
| Triglycerides (mmol/L)   | 2.67 ± 2.12            | 2.45 ± 1.66                                | 2.87 ± 2.46                                | NS (P = 0.30)                                |
| LDL cholesterol (mmol/L) | 2.54 ± 0.85            | 2.63 ± 0.83                                | 2.45 ± 0.87                                | NS (P = 0.27)                                |
| HDL cholesterol (mmol/L) | 1.04 ± 0.32            | 1.06 ± 0.36                                | 1.03 ± 0.28                                | NS (P = 0.60)                                |
| Hematocrit               | 41.9 ± 5.2             | 42.4 ± 3.3                                 | 41.8 ± 3.6                                 | NS (P = 0.30)                                |
| GFR (mL/min/1.73 m²)     | 91.7 ± 18.8            | 90.6 ± 19.5                                | 92.9 ± 18.2                                | NS (P = 0.49)                                |
| Albumin (g/L)            | 36.9 ± 3.6             | 37.1 ± 3.7                                 | 36.7 ± 3.6                                 | NS (P = 0.45)                                |
| Protein (g/L)            | 72.9 ± 8.6             | 72.1 ± 10.5                                | 73.1 ± 6.1                                 | NS (P = 0.78)                                |
| CRP (mg/L)               | 5.05 ± 3.16            | 4.72 ± 3.06                                | 5.32 ± 3.20                                | NS (P = 0.31)                                |
| Body fat mass (kg)       | 39.7 ± 13.9            | 30.4 ± 8.4                                 | 49.8 ± 11.4                                | <0.0001                                       |
| Body free-fat mass (kg)  | 58.2 ± 11.8            | 56.9 ± 11.4                                | 63.3 ± 13.1                                | NS (P = 0.08)                                |
| Liver fat content (%)    | 17.1 ± 11.5            | 14.1 ± 10.5                                | 20.0 ± 11.7                                | 0.009                                         |
| Visceral fat area (cm²)  | 263 ± 124              | 230 ± 96                                   | 303 ± 140                                  | 0.004                                         |
| Subcutaneous fat area (cm²) | 404 ± 160             | 330 ± 119                                  | 485 ± 162                                  | <0.0001                                       |
| Visceral/subcutaneous fat area ratio | 0.80 ± 0.63 | 0.84 ± 0.67                                | 0.77 ± 0.64                                | NS (P = 0.61)                                |
| Glycation gap (G-Gap) (%)| 0.12 ± 1.67            | −0.27 ± 1.04                               | 0.51 ± 2.05                                | 0.013                                         |

BMI, body mass index; CRP, C-reactive protein; GFR, glomerular filtration rate; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NS, not significant. The bold values represent significant P values.
In multivariate analysis, the independent predictors for fructosamine were HbA1c ($b = 0.539$, $P < 0.0001$) and BMI ($b = -0.210$, $P = 0.028$), whereas age, sex, albumin, subcutaneous fat and liver fat were not. When fat mass was introduced into the statistical model, the independent predictors for fructosamine were HbA1c ($b = 0.582$, $P < 0.0001$) and fat mass ($b = -0.245$, $P = 0.01$), whereas BMI was no longer associated with fructosamine.

**Fat mass and fructosamine**

To obtain further insight into the association between fructosamine and body fat mass, the patients were then divided into two groups according to body fat mass, above or below the median. The correlation coefficient between fructosamine and HbA1c was stronger in patients with fat mass below the median ($r = 0.687$) than in those with fat mass above the median ($r = 0.440$). The slope of the regression line between fructosamine and HbA1c was significantly steeper in patients with body fat mass below the median ($y = 29.415x + 145.52$) than above the median ($y = 15.167x + 262.24$), and the slopes of the two regression lines were significantly different ($P = 0.029$).

In addition, the G-Gap was significantly higher in patients with fat mass above the median than below the median ($0.52 \pm 1.97$ vs $-0.25 \pm 1.23$, $P = 0.014$), as shown in Figure 1c, and positive, indicating underestimation of the true HbA1c with fructosamine.

Furthermore, we carried out an additional analysis considering total fat area (visceral fat + subcutaneous fat). We found that in the patients with total fat area below the median value, the correlation coefficient between fructosamine and HbA1c

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**Figure 1** | Influence of obesity on the association between fructosamine and glycated hemoglobin (HbA1c). (a) Regression line between fructosamine and HbA1c in a type 2 diabetes patient with body mass index (BMI) below the median (open square, unbroken line) and in a type 2 diabetes patient with BMI above the median (full square, dashed line). (b) Glycation gap in type 2 diabetes patients with BMI below the median (grey) and in type 2 diabetes patients with BMI above the median (black). (c) Glycation gap in type 2 diabetes patients with body fat mass below the median (grey) and in type 2 diabetes patients with body fat mass above the median (black).
was stronger \((r = 0.685)\) than in those with total fat area above the median value \((r = 0.460)\). The slope of the regression line between fructosamine and HbA1c was significantly steeper in patients with total fat area below the median \((y = 29.17x + 148.26)\) than above the median \((y = 17.61x + 239.19)\).

**DISCUSSION**

We show in the present study that the association between fructosamine and HbA1c is significantly reduced in the most obese type 2 diabetes patients, and that increased body fat mass is the main factor responsible for this reduced association, whereas liver fat content or fat distribution (subcutaneous, visceral) do not seem to be involved.

Fructosamine has been shown to be affected by some situations, such as macroproteinuria or renal insufficiency\(^{14,15}\), and influenced by plasma albumin\(^{16}\). In the present study, patients with macroproteinuria or renal insufficiency were excluded. Furthermore, plasma albumin was normal in our patients, and albumin was not shown to influence fructosamine both in univariate or multivariate analyses.

Because obesity is increasingly frequent among type 2 diabetes patients, it is important to analyze whether the association between fructosamine and HbA1c is modified by obesity. Previous studies, carried out with first-generation fructosamine assays, have shown conflicting results in obese individuals. A reduced association between HbA1c and fructosamine in obese individuals has been reported in two studies\(^6\,7\), but not in another study\(^8\). In addition, in those studies, only obese individuals without diabetes were studied in two of the studies\(^6\,7\), and the number of obese patients with diabetes was very limited in the third study\(^8\). In our present study, using a more recent fructosamine assay, we showed in a population of 112 type 2 diabetes patients that the association between fructosamine and HbA1c was significantly reduced in the more obese patients. We found that the more obese type 2 diabetes patients have a higher G-Gap with a positive value, indicating that fructosamine underestimates HbA1c. The correlation between fasting blood glucose and HbA1c was not significantly different between the more obese and the other type 2 diabetes patients, arguing that it is fructosamine and not HbA1c that is modified by obesity.

Furthermore, we show that increased fat mass is the factor driving the reduced association between fructosamine and HbA1c in the more obese patients. Liver fat content and fat distribution do not appear to influence the association between fructosamine and HbA1c. The G-Gap was significantly higher and positive in the patients with body fat mass above the median, signifying underestimation of HbA1c by fructosamine.

Fructosamine reflects overall glycemic level during a 2–3-week period including postprandial variation. The influence of BMI on postprandial glucose variability in type 2 diabetes patients is not totally clarified. It has been shown in newly diagnosed Chinese type 2 diabetes patients that individuals with BMI <24 had increased glycemic variability compared with those with BMI >24\(^7\). However, in other studies, BMI has not been shown to significantly influence glycemic variability\(^18\,19\).

In our present study, we do not believe that postprandial glucose levels were different between patients with BMI below the median and above the median, because fasting blood glucose as well as fructosamine and HbA1c, which embrace post-prandial glucose levels, were not different between the two groups.

The reasons for this reduced association between fructosamine and HbA1c in type 2 diabetes patients with markedly increased body fat mass are unknown. It has been shown in vitro that incorporation of \(^14\)C-glucose in serum proteins was reduced in obese individuals, and that the rate of formation of fructosamine was slower in obese individuals compared with lean individuals\(^8\). How increased fat mass could reduce protein glycation and fructosamine formation is unclear. It has been shown that the local environment around the proteins has a direct effect on the amino group’s reactivity with glucose\(^20\). The rate of glycation of albumin in vitro is enhanced when fatty acids are removed from the albumin, and we might suppose that excess of fatty acids, promoted by increased fat mass, could cover some amino groups of the albumin and thus reduce its glycation. Further studies are required to clarify this point.

Although fructosamine is different from glycated albumin, because it is a marker of protein glycation, it predominantly measures glycated albumin, as albumin is the most abundant of serum proteins. Because the rate of glycation of albumin is ninefold greater than that of hemoglobin\(^20\), albumin is likely to be more sensitive to factors influencing glycation than hemoglobin. This might explain why fructosamine appears to be affected by increased fat mass, but not HbA1c.

The present study had some limitations. First, it was carried out with white patients, and the results might not be extrapolated to other populations. Second, the present study was limited to 112 type 2 diabetes patients, and our results need to be confirmed in a larger-scale study.

**DISCLOSURE**

The authors declare no conflict of interest.

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