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Percentage of body fat and plasma glucose predict plasma sialic acid concentration in type 2 diabetes mellitus

Nicola A. Englyst[a,*], Martin A. Crook[b], Peter Lumb[b], Anna J. Stears[c], Michael G. Masding[c,d], Stephen A. Wootton[c], Derek D. Sandeman[d], Christopher D. Byrne[a]

[a]*Endocrinology & Metabolism Unit, University of Southampton, Southampton SO16 6YD, UK*
[b]*Chemical Pathology Department, St Thomas' Hospital, London SE1 9RT, UK*
[c]*Institute of Human Nutrition, University of Southampton, Southampton SO16 6YD, UK*
[d]*Diabetes and Endocrinology, University of Southampton and Southampton Hospitals Trust, Southampton SO16 6YD, UK*

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Abstract

Circulating sialic acid is an independent risk factor for cardiovascular disease and is higher in people with type 2 diabetes mellitus. Sialic acid is associated with body mass index, but it is uncertain whether body fat contributes to the higher levels of sialic acid in type 2 diabetes mellitus. Therefore, we have investigated whether the higher levels of sialic acid observed in type 2 diabetes mellitus persist when controlling for fatness. Fasting plasma samples were collected from 24 individuals with type 2 diabetes mellitus and 24 controls. Percentage of body fat was measured by bioelectrical impedance. Plasma sialic acid was quantified by an enzymatic method. Plasma sialic acid was higher in the group with type 2 diabetes mellitus than controls (602 ± 14 vs 545 ± 14 mg/L, $P = .007$). Percentage of body fat was associated with plasma sialic acid concentration in both the control group ($r = 0.481$, $P = .020$) and the group with type 2 diabetes mellitus ($r = 0.527$, $P = .007$). Fasting glucose was also associated with plasma sialic acid in the group with type 2 diabetes mellitus ($r = 0.700$, $P < .001$). Adjustment for percentage of body fat accounted for the higher levels of sialic acid in type 2 diabetes mellitus. Using linear regression, 54.3% of the variation of plasma sialic acid was explained by percentage of body fat and glucose concentrations in the whole group. Seventy-four percent of sialic acid variation was explained by the same model in type 2 diabetes mellitus. In conclusion, this is the first study to show that percentage of body fat predicts plasma sialic acid concentration and contributes toward higher levels of sialic acid in type 2 diabetes mellitus.

1. Introduction

Epidemiologic studies suggest that circulating sialic acid concentration is an independent predictor for both cardiovascular and cerebrovascular events [1,2]. In addition, patients with cardiovascular disease (CVD) [3,4] and acute myocardial infarction [4,5] have increased serum sialic acid concentrations. Sialic acid is higher in people with type 2 diabetes mellitus than controls [6-10], and higher levels of circulating sialic acid precede the development of type 2 diabetes mellitus and insulin resistance [11].

The mechanisms by which sialic acid is linked to type 2 diabetes mellitus and CVD are unclear. One potential mechanism is through obesity because type 2 diabetes mellitus is often associated with higher body mass index (BMI; weight in kilograms divided by the square of height in meters). A number of studies have demonstrated a positive correlation between sialic acid and BMI [12-14]. However, BMI is affected by both lean and fat mass, and, therefore, it is unclear whether body fat is the component of BMI that is responsible for the relationship with sialic acid. To date, no direct comparisons have been made of body fat with sialic acid.

The source of plasma sialic acid is unclear. Some of the sialic acid in the circulation comes from the diet, and, therefore, it has been suggested that concentrations may reflect metabolic status, body tissue levels, and nutritional status [15]. Most of the sialic acid in plasma and serum is associated with lipoproteins, although some of the circulating sialic acid may result from cellular cleavage because all

* Corresponding author. Endocrinology & Metabolism Unit, Southampton General Hospital, SO16 6YD Southampton, UK. Tel.: +1 023 8079 6925; fax: +1 023 8079 4945.
E-mail address: nae@soton.ac.uk (N.A. Englyst).

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tissues have the capacity to synthesize sialic acid, and many cells also express neuraminidases that cause cleavage of sialic acid from the cell surface. Interestingly, leukocytes from people with type 2 diabetes mellitus and models of diabetes mellitus have been reported to have increased levels or activities of neuraminidases, as well as decreased cellular sialic acid [16-18].

Because people with type 2 diabetes mellitus often have a higher BMI than controls, it is plausible that the higher levels of sialic acid observed in type 2 diabetes mellitus are due to higher BMI, particularly higher percentage of body fat in people. Therefore, we have compared plasma sialic acid concentration in BMI-matched individuals with type 2 diabetes mellitus and controls and extended these observations to consider the effect of percentage of body fat.

2. Research design and methods

Volunteers (n = 48) gave written informed consent for the study, approved by Southampton and South West Hampshire local research ethics committees. This cohort included 24 people with type 2 diabetes mellitus (12 men and 12 women) and 24 people without type 2 diabetes mellitus (controls, 12 men and 12 women) (mean age, 57.3 ± 8.3 years). Diabetes was defined using standard World Health Organization criteria and type 2 diabetes mellitus assigned if the age of onset was older than 40 years and they were on oral hypoglycemics or diet alone for more than 2 years. People with type 2 diabetes mellitus were recruited from a hospital clinic (controlled by diet or oral hypoglycemics), whereas controls were recruited from the community via advertising. All women were postmenopausal (some of these volunteers were a subgroup of the postmenopausal female volunteers described by Masding et al [19]). Postmenopausal status was verified by amenorrhea for more than 1 year and elevated gonadotrophins. No subjects were known to have CVD. Volunteers avoided strenuous exercise and alcohol for 2 days before the study and were given a standard meal on the evening before the study. Subjects were excluded if on lipid-lowering therapy, and all volunteers were asked to avoid medication (including oral hypoglycemic agents) from the evening before the study. Volunteers were asked to fast overnight and the study began at 08:00 AM. Blood samples were collected into citrate, from a cannulated vein.

Data on anthropometric measurements (age, weight, height, BMI, waist circumference, hip circumference, and percentage of body fat) were collected (BodyStat bioelectrical impedance was recorded as a measure of percentage of body fat). Serum triglycerides, insulin, and glucose were measured using standardized laboratory techniques as described in reference [19]. Plasma sialic acid was quantified using an enzymatic method (Boehringer Mannheim, Lewes, Sussex, UK) on a Roche Cobas Fara analyzer (Roche, Basel, Switzerland) [20].

2.1. Statistical analysis

Data are presented as arithmetic mean ± SD. Data for triglyceride and insulin concentration were normalized by logarithmic transformation (presented as median and 95% confidence intervals [CIs]). Differences between controls and people with type 2 diabetes mellitus were analyzed by unpaired Student *t* tests. Associations between sialic acid concentration and other parameters were examined using Pearson correlations. The interactions between type 2 diabetes mellitus, sex, BMI, and percentage of body fat were examined using analysis of covariance. Independent predictors of plasma sialic acid concentration were determined using multiple linear regression with a stepwise model. A P value of less than .05 was considered to be significant.

3. Results

3.1. Cohort characteristics and associations of plasma sialic acid with measures of body composition

Characteristics of the cohorts of individuals with type 2 diabetes mellitus and controls are given in Table 1. There were no significant differences in BMI (P = .286), percentage of body fat (P = .527), waist circumference (P = .081), waist-to-hip ratio (P = .191), age (P = .345), or fasting insulin concentration (P = .324) between the group with type 2 diabetes mellitus and controls. As expected, fasting triglycerides (P = .002), nonesterified free fatty acids (NEFAs) (P = .001), and glucose (P < .001) were different between groups.

Despite matching the groups for BMI, the plasma sialic acid concentration was still 57 mg/L higher in the group with type 2 diabetes mellitus than in the control group (P = .007).

In the whole group, plasma sialic acid concentration was associated with percentage of body fat (r = 0.426, P = .001; Fig. 1A), but not with BMI or waist circumference. Plasma

Table 1
Cohort characteristics

Parameter	Controls (n = 24)	Type 2 diabetes mellitus (n = 24)	*P*
Age (y)	55.2 ± 8.3	57.6 ± 8.4	.345
BMI (kg/m^2)	29.2 ± 4.0	30.5 ± 4.1	.286
Percentage of body fat (%)	33.4 ± 8.2	35.0 ± 1.8	.527
Waist circumference (cm)	100 ± 9	106 ± 12	.081
Waist-to-hip ratio	0.9 ± 0.2	1.0 ± 0.3	.191
Triglycerides (mmol/L)	1.6 (0.54-2.3)	2.4 (0.52-2.3)	.002
NEFAs (mmol/L)	145 ± 35	202 ± 13	.001
Glucose (mmol/L)	5.5 ± 0.5	10.6 ± 2.6	<.001
Insulin (μU/mL)	7.9 (6.8-14.6)	12.88 (9.9-16.8)	.324
Sialic acid (mg/L)	545 ± 75	602 ± 74	.007

Data are expressed as mean ± SD or median (95% CI).

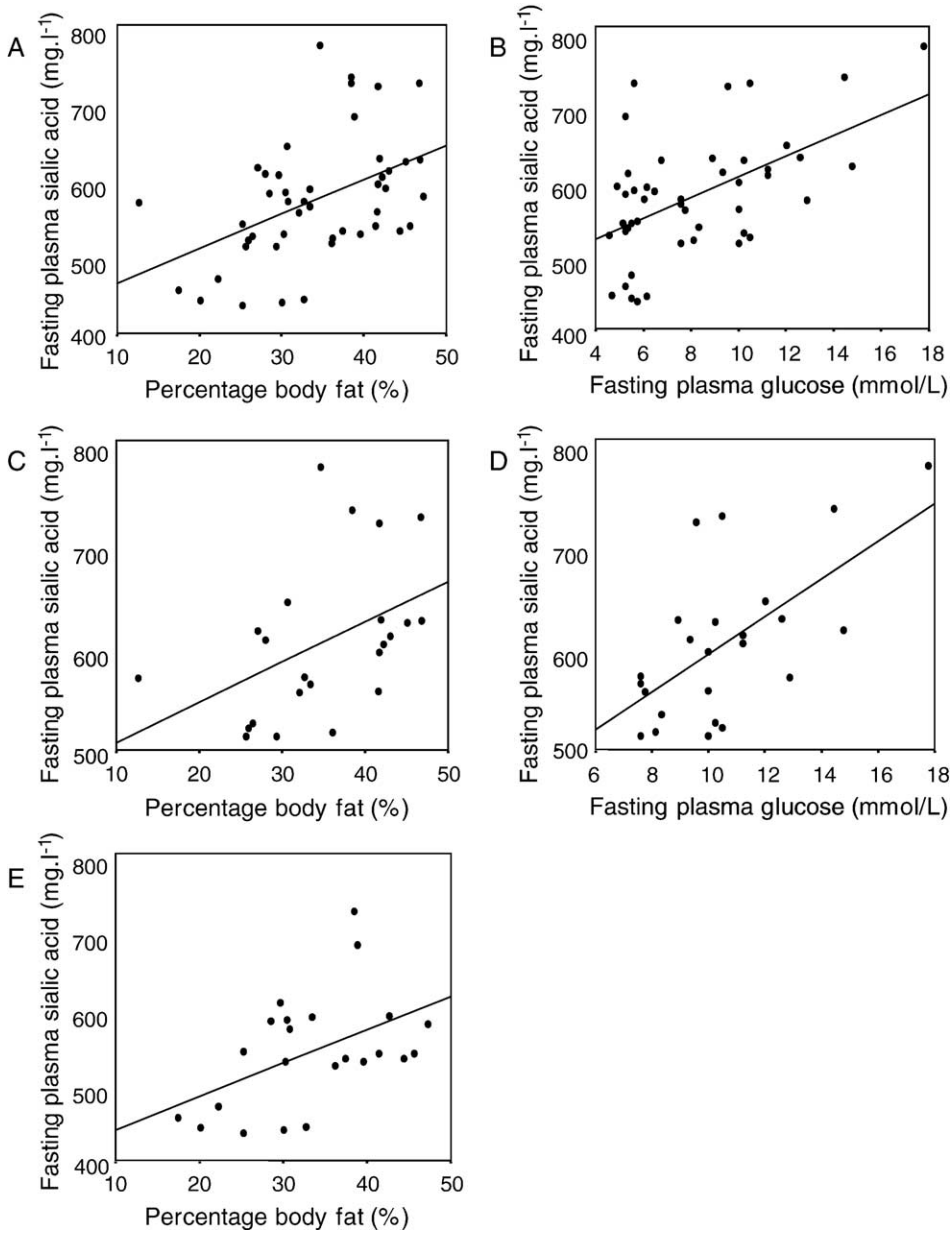

Fig. 1. Correlation of plasma sialic acid with (A) percentage of body fat in the whole group, (B) plasma glucose in the whole group, (C) percentage of body fat in people with type 2 diabetes mellitus, (D) plasma glucose in people with type 2 diabetes mellitus, and (E) percentage of body fat in the control group.

sialic acid concentration was also correlated with fasting glucose ($r = 0.546$, $P < .001$; Fig. 1B), triglycerides ($r = 0.430$, $P = .001$), and NEFAs ($r = 0.551$, $P < .001$).

Individuals with type 2 diabetes mellitus also showed an association between plasma sialic acid and percentage of body fat ($r = 0.527$, $P = .007$; Fig. 1C). In addition, they showed correlations between plasma sialic acid and glucose ($r = 0.700$, $P < .001$; Fig. 1D), triglycerides ($r = 0.627$, $P = .001$) and NEFAs ($r = 0.649$, $P < .001$). In controls, there was a significant correlation between plasma sialic acid and percentage of body fat ($r = 0.481$, $P = .020$; Fig.1E), but not with any other measures of body composition or biochemical parameters.

3.1.1. Is plasma sialic acid higher in type 2 diabetes mellitus than controls and is sialic acid normalized by correcting for percentage of body fat?

There was no statistically significant interaction between sex and type 2 diabetes mellitus in determining plasma sialic acid concentration. However, at the univariate level, individuals with type 2 diabetes mellitus had higher plasma sialic acid than controls (602 ± 14 vs 545 ± 14 mg/L, $P = .007$) despite the fact that the groups were BMI-matched groups. Additional post hoc analysis to control for BMI still resulted in higher levels of sialic acid in people with type 2 diabetes mellitus compared with controls ($P = .012$). After correction for percentage of body fat to 34.2% for the whole

group the difference in sialic acid concentration with type 2 diabetes mellitus persisted, although it was no longer statistically significantly different (618 ± 28 mg/L for type 2 diabetes mellitus vs 571 ± 25 mg/L for controls, $P = .214$). There was no significant difference in plasma sialic acid concentration with medication (sulfonylurea, n = 7; metformin, n = 6; glitazone, n = 1; angiotensin-converting enzyme inhibitor, n = 2; β-blocker, n = 2; or other medication, n = 22). These data suggest that percentage of body fat accounts at least partly for the higher levels of sialic acid seen with type 2 diabetes mellitus.

Women had higher levels of plasma sialic acid than men (603 ± 14 vs 544 ± 14 mg/L, $P = .005$). Differences in sialic acid between women and men were also lost upon correction for percentage of body fat (613 ± 30 for women vs 579 ± 22 mg/L, for men, $P = .411$). This suggests that percentage of body fat accounts at least partly for the higher levels of sialic acid seen with female sex.

3.2. Best model for predicting fasting plasma sialic acid

In the whole group, the best model predicted 54.3% of the variation in fasting sialic acid concentration. Fasting glucose (β coefficient, 0.607; $P < .001$; 95% CI, 0.926-2.203) and percentage of body fat (β coefficient, 0.396; $P = .003$; 95% CI, 0.149-0.649) were both independent predictors of fasting sialic acid concentration. In controls, the best model was composed only of percentage of body fat (β coefficient, 0.484; $P = .020$; 95% CI, 0.075-0.792) and explained 23.1% of the variation in plasma sialic acid. In type 2 diabetes mellitus, the best model consisted of glucose (β coefficient, 0.530; $P = .001$; 95% CI, 0.734-2.367), percentage of body fat (β coefficient, 0.466; $P = .001$; 95% CI, 0.184-0.641), and triglycerides (β coefficient, 0.331; $P = .023$; 95% CI, 1.717-20.491). This model explained 73.9% of the variation in plasma sialic acid.

4. Discussion

This study is the first to demonstrate that percentage of body fat is an independent predictor of fasting plasma sialic acid and that sialic acid concentration is higher in type 2 diabetes mellitus, despite matching BMI in the groups by a priori study design and in post hoc analysis. This difference in sialic acid was of a similar magnitude to other cross-sectional studies of people with type 2 diabetes mellitus and controls that do not take into account BMI. The differences in sialic acid in type 2 diabetes mellitus remain, although they are no longer statistically significant after adjustment for percentage of body fat. This suggests that percentage of body fat may contribute to the higher levels of plasma sialic acid observed in people with type 2 diabetes mellitus, although other factors may also be important.

The major strengths of this study are that the groups are well characterized and that percentage of body fat has been measured in addition to BMI and weight. One of the potential criticisms of this study is that the difference in sialic acid concentration in type 2 diabetes mellitus compared with controls is relatively small, although it is consistent with the published literature. However, in support of the notion that this variation may be physiologically important, an increase in 100 mg/L of sialic acid is associated with a change in absolute width of coronary segments, after adjustment for age, blood pressure, smoking, and low-density lipoprotein cholesterol [21].

The data from our study advance current understanding of the relationship between body size and circulating sialic acid concentration because previous studies have only demonstrated that increased BMI and waist measurements are correlated with sialic acid concentration without any measurement of body composition [2,12-14]. Our data on percentage of body fat suggest that sialic acid concentrations may be related to adipose tissue rather than to total body mass or lean mass, although the mechanisms may be different depending on type 2 diabetes mellitus status and sex. It has recently been demonstrated that sialic acid concentration in women predicts the number of features of the metabolic syndrome with a proinflammatory phenotype, regardless of BMI [22].

Type 2 diabetes mellitus is associated with increased obesity, inflammation, and sialic acid concentrations [9-11], and in a large study of more than 12 000 people, the odds ratio for developing type 2 diabetes mellitus was 3.7 with sialic acid concentration greater than the median concentration [11]. However, our study is the first study to investigate the importance of fatness in determining plasma sialic acid levels in type 2 diabetes mellitus. Our data, when adjusted for percentage of body fat, suggests that fatness at least partly determines the higher levels of plasma sialic acid in type 2 diabetes mellitus. We have also demonstrated that percentage of body fat is an independent predictor for plasma sialic acid in both controls and people with type 2 diabetes mellitus.

There are several possible mechanisms by which adipose tissue, glucose, and sialic acid concentration may be connected. Given that obesity is associated with a chronic inflammatory state and adipose tissue has been shown to secrete several proinflammatory molecules, it is plausible that sialic acid is increased as part of, or in response to, a chronic inflammatory state associated with obesity. Sialic acid may also contribute to obesity because desialylation of adipocytes (and therefore presumably increased circulating sialic acid) leads to a reduction in insulin action associated with insulin resistance [23,24]. Similarly, alterations in insulin sensitivity due to sialic acid may form the link between sialic acid and glucose. Further work is now required to investigate the mechanisms by which glucose is related to sialic acid. Research is also required to demonstrate that reduction of plasma sialic acid levels occurs with reversal of hyperglycemia because previous research has shown that treatment of people with type 2 diabetes mellitus

with insulin or oral hypoglycemic drugs has been shown to restore cell-associated sialic acid and sialidase to the same levels as found in controls [16].

It is also not clear whether increased levels of serum/plasma sialic acid is associated with increased risk of atherosclerosis because of the elevated levels in the circulation or because increased serum/plasma sialic acid represents changes in sialic acid on cell surfaces that cause changes in cell function or activity. In support of the latter suggestion, sialic acid was reduced in leukocytes from people with type 2 diabetes mellitus compared with controls, whereas sialidase activity was increased [16]. Treatment with insulin or oral hypoglycemic drugs restored cellular sialic acid and sialidase to the same levels as in controls. Furthermore, it has been demonstrated that desialylation of adipocytes and hepatocytes leads to a reduction in insulin action associated with insulin resistance [23-25], whereas removal of sialic acid from hepatocytes reduces insulin-stimulated lipogenesis [25]. It has been demonstrated that the activity of sialidases (the enzyme that liberates sialic acid) is increased in human leukocytes from people with diabetes mellitus [16], in endothelial cells stimulated with advanced glycation endproducts [18], and in an animal model of diabetes [17]. Additional research is required to elucidate the mechanisms linking adipose with circulating sialic acid.

In summary, this study has demonstrated that percentage of body fat is an independent predictor of fasting plasma sialic acid. We have demonstrated that sialic acid concentration is higher in BMI-matched groups of individuals with type 2 diabetes and controls. In addition, the results from this study suggest that percentage of body fat may contribute to the higher levels of plasma sialic acid observed in people with type 2 diabetes mellitus, although other factors may also be important.

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