Detection of Sialic Acids in Adipose Tissue of Adventist Health Study-2 Participants with Different Dietary Patterns

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Introduction

The application of glycobiology to human medicine is a relatively new field of study. Of special interest in this field are sialic acids (Sias), which are a family of molecules derived from neuraminic acid, a nine-carbon parent sugar. Generally, Sias occur at the ends of carbohydrate chains, either attached to cellular surfaces or as secreted glycoconjugates like glycoproteins or glycolipids. Given their position and structural diversity, Sias modulate a wide variety of biological processes associated with health and disease.

The most abundant Sias in mammals are N-Acetylneuraminic acid (Neu5Ac) and its hydroxylated derivative form N-glycolylneuraminic acid (Neu5Gc) and 2-keto-3-deoxy-D-glycero-D-galactose-nonionic acid (KDN). There is evidence that the abundance of these Sias may be altered with the onset or progression of chronic diseases such as cardiovascular disease, diabetes, or cancer. Interestingly, Sias may be ingested from animal sources in the diet and enter body fluids or become metabolically incorporated into tissues, regulating inflammation or other disease processes. However, very little is known about the concentration of Sias in adipose tissue and the implications for human health. To explore the meaning of Sias in human adipose tissue, it is first necessary to develop a method for the extraction, detection, and quantification of Sias, particularly from individuals following different dietary patterns.

Objectives

The objectives of this study were to 1) develop a method to extract, detect, and quantify KDN, Neu5Gc, and Neu5Ac from adipose tissue, and 2) compare the abundance of these Sias in Adventist Health Study-2 participants following habitual, long-term vegetarian and non-vegetarian dietary patterns. Furthermore, the content of Sias from adipose tissue in animals was assessed to verify the ability of the developed method to detect Sias in other biological sources. This was done using LC-MS/MS, given its sensitivity and specificity for the analysis of Sias.

Methods

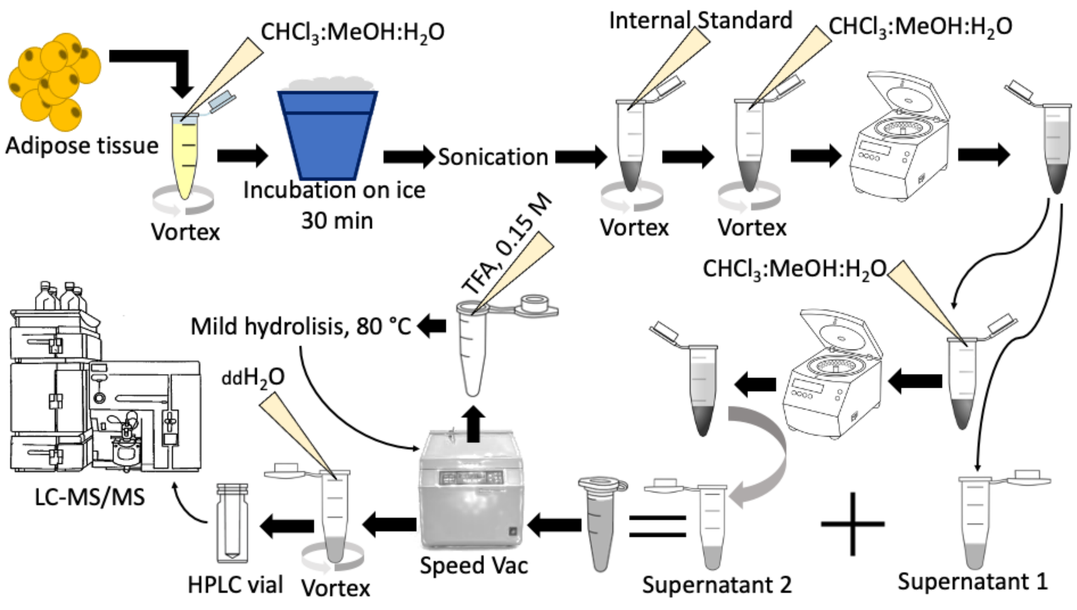

Fig 1. The developed method for the extraction, detection, and quantification of sialic acids from adipose tissue using LC-MS/MS.

Results

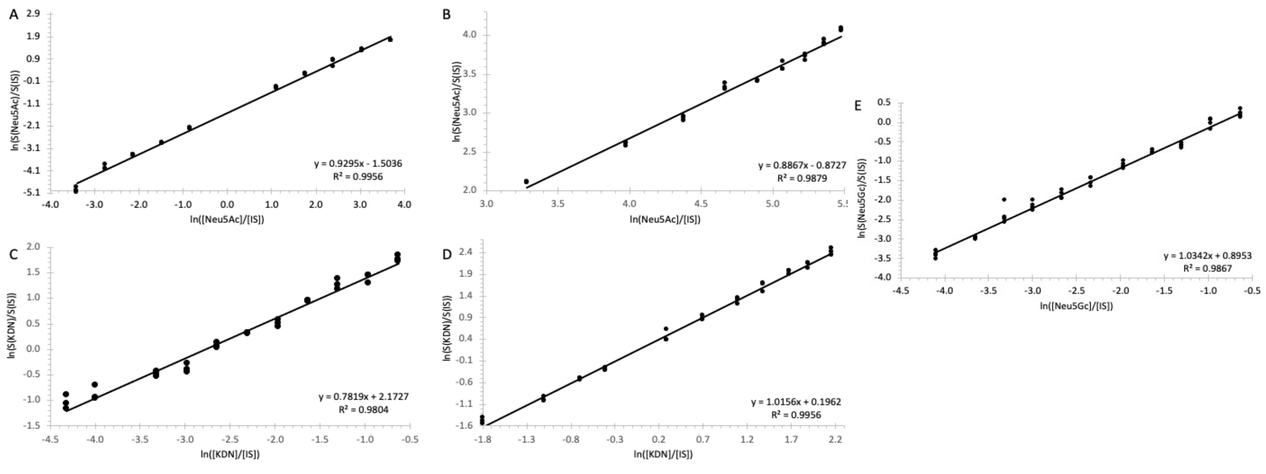

Figure 2. Quantitative calibration curves showing linearity and sensitivity of the LC-MS/MS method for detection of the concentrations of Neu5Ac (A-B), KDN (C-D), and Neu5Gc (E).

Results

Table 1. Content of Sialic acids in animal adipose tissue (µg/mL).

Species; *n*=3 (each)	Neu5Ac		KDN		Neu5Gc		Total
Chicken	2.176	±0.37	0.262	±0.04	-	-	2.438
Pork	4.68	±0.39	0.324	±0.02	0.665	±0.03	5.669
Lamb	2.78	±0.33	0.276	±0.06	0.536	±0.04	3.592
Cow	3.128	±0.56	0.105	±0.10	1.725	±0.16	4.958

- : No detection

Table 2. Demographic characteristics of study population[1]

	Lacto-ovo	Non-vegetarian	Vegan	P[2]
Participants	59 (36.6)	50 (31.1)	52 (32.3)	
Age	57.2 (13.2)	63.2 (12.9)	61.3 (14.3)	0.08
Sex				
Female	43 (37.4)	37 (32.2)	35 (30.4)	
Male	16 (44.4)	13 (36.1)	17 (47.2)	0.78
Race				
Black	44 (41.5)	39 (36.8)	23 (21.7)	
non-Black	15 (27)	11 (0.2)	29 (0.53)	0
Substudy				
ARHS1	14 (0.35)	17 (42.5)	9 (0.23)	
Pilot	45 (0.37)	33(0.27)	43 (0.35)	0.14
BMI	30.9 (7.4)	32.4(7.5)	27.6 (5.5)	0

[1]Values are presented as n (%), or mean (SD).
[2]P value computed using chi-square test for categorical variables and t test for continuous variables

Table 3. Adjusted means from linear regression model of associations of dietary patterns with sialic acids[1]

	Vegan	Non-vegetarian	P
Neu5Ac (units)	18.0	10.9	0.04
KDN	0.7	0.6	0.8
	Vegan	Lacto-ovo	P
Neu5Ac	18.0	14.6	0.3
KDN	0.8	0.8	0.6
	Lacto-ovo	Non-vegetarian	P
Neu5Ac	13.3	9.2	0.1
KDN	0.6	0.5	0.3

[1]Linear regression model adjusted for age at blood collection, race, gender, substudy, batch

Table 4. Associations of BMI with sialic acids[1]

	β coefficient	Std. Err	P value
KDN	-0.02	0.01	0.03
Neu5Ac	-0.02	0.01	0.16

[1]Values estimated from linear regression model adjusted for age at blood collection, substudy, race, gender, diet group, batch

Discussion

- It has been recently suggested that high levels of Sias may be associated with mechanisms to counteract atherosclerosis. Hence, vegetarians may have greater protection against atherosclerosis and cardiovascular disease.
- It is possible that the diet composition affects *de novo* synthesis of Neu5Ac, KDN, and other Sias in adipose tissue.
- BMI was inversely associated with Sias, and may partially mediate the effect of diet.
- The non-human meat-derived Neu5Gc was not detected in human adipose tissue. Either Neu5Gc is not present in human adipose tissue or the concentration of this monosaccharide is undetectable with our method and the LC-MS/MS equipment used in our study.

Conclusion

Neu5Ac and KDN were extracted, detected, and quantified in human adipose tissue from vegetarian and non-vegetarian participants of the AHS-2. Vegans and non-vegetarians showed statistical differences in the concentration of Neu5Ac, an association that is likely to be influenced by BMI. The lower concentration of Sias in adipose tissue seen in non-vegetarians or with higher BMI might help explain the increased risk of cardiovascular diseases (atherosclerosis, etc.) for these individuals. The developed method furthermore allowed the detection of Neu5Ac, KDN, and Neu5Gc in adipose tissue of animals expected to have these Sias.

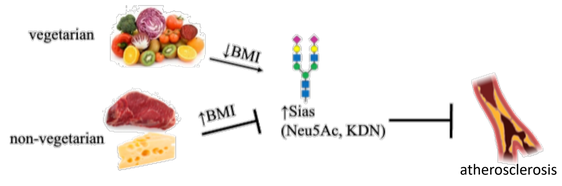

Fig 3. Theoretical relationship between diet, BMI, Sias, and CVD.

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