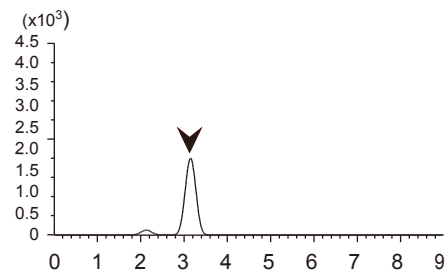
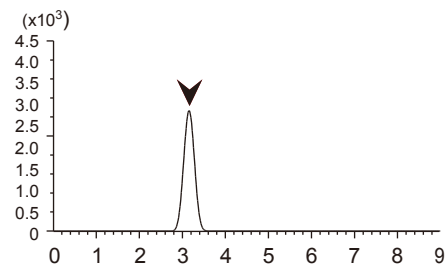


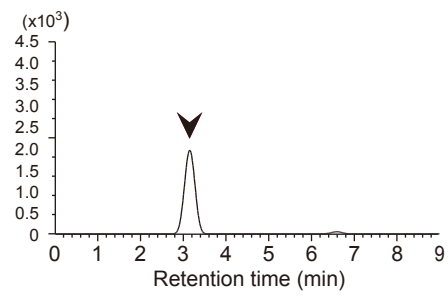
A

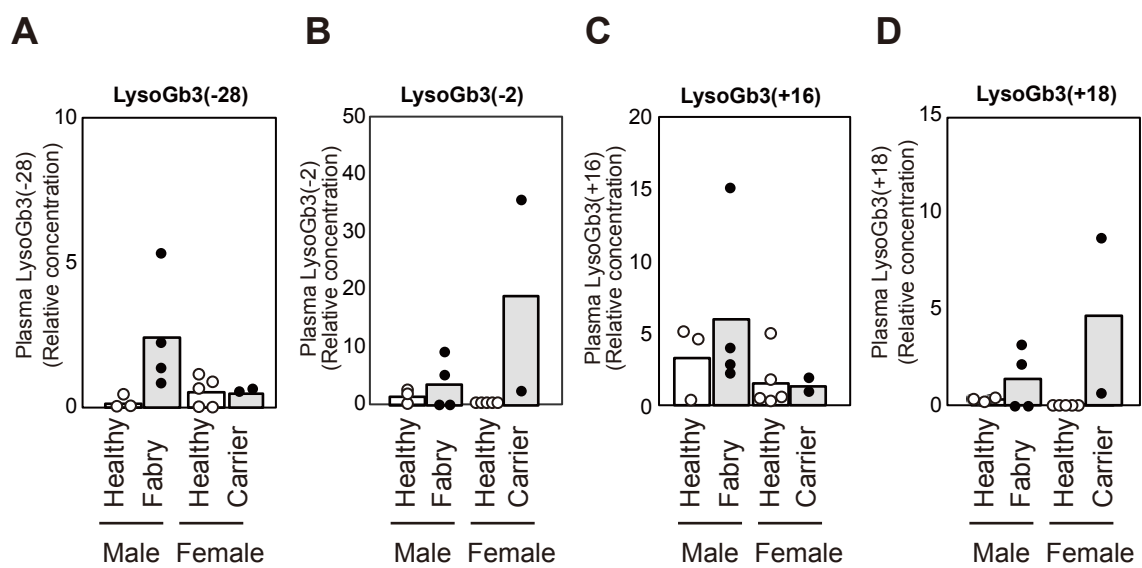


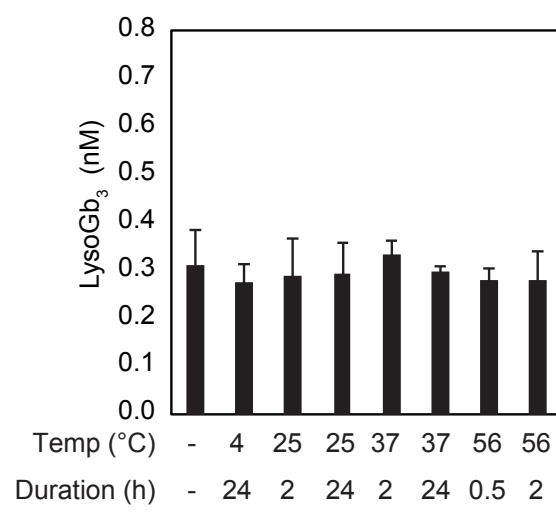
B



C







Supplementary Fig. 3

Ohira M et al

Supplementary Figure Legends

Supplementary Fig. 1

Chromatograms for LysoGb₃(+18) (LCB = t18:0) in FBS of separate lot. (A) A chromatogram for LysoGb₃(+18) in FBS-2. (B) A chromatogram for LysoGb₃(+18) in FBS-3. (C) A chromatogram for LysoGb₃(+18) in FBS-4. Other details were described in Supplementary Table 1.

Supplementary Fig. 2

Accumulation of LysoGb₃ species in human plasma. (A) Changes in LysoGb₃(-28) (LCB = d16:1) in male healthy controls, male Fabry patients, female healthy controls, and female carriers. (B) Changes in LysoGb₃(-2) (LCB = d18:2) in male healthy controls, male Fabry patients, female healthy controls, and female carriers. (C) Changes in LysoGb₃(+16) (LCB = t18:1) in male healthy controls, male Fabry patients, female healthy controls, and female carriers. (D) Changes in LysoGb₃(+18) (LCB = t18:0) in male healthy controls, male Fabry patients, female healthy controls, and female carriers. The number for subjects was: male healthy controls ($n = 3$); male Fabry patients ($n = 4$); female healthy controls ($n = 5$); female carriers ($n = 2$), respectively.

Supplementary Fig. 3

Stability of LysoGb₃ in pooled human plasma under various storage conditions at 37°C. Data were from mean $\pm$ SD ($n = 3$).

Supplementary Table 1 Source and origin of country for pooled human plasma and FBS used in this study.

Sample	Supplier (Country)	Cat#	Lot#	Origin of country
Pooled human plasma	Kohjin Bio (Japan)	12250210	BJ9771A	United States
FBS-1	Nichirei (Japan)	171012	9E0808	Australia
FBS-2	GIBCO (US)	10270-106	4240183K	Brazil
FBS-3	GIBCO (US)	10270-106	4240584K	Brazil
FBS-4	Biowest (France)	S1820-500	S18092S1530	Columbia

Supplementary Table 2 MS1, MS2, cone energy and collision energy for the quantification of LysoGb₃ and its analogues in human plasma using LC-MS/MS^a.

	MS1	MS2	Cone	Collision	Confirmed/Suggested compound	Putative fragmentation for MS2
	(<i>m/z</i>)	(<i>m/z</i>)	(V)	(V)		
LysoGb ₃	786.4	282.2	20	40	LysoGb ₃ (d18:1)[LCB-H ₂ O+H] ⁺	
LysoGb ₃ -Gly (IS)	843.4	264.2	30	45	LysoGb ₃ (d18:1-[LCB-2×H ₂ O+H] ⁺ N-Gly)	
LysoGb ₃ (-28)	758.4	254.3	20	40	LysoGb ₃ (d16:1)[LCB-H ₂ O+H] ⁺	
LysoGb ₃ (-12)	774.4	252.3	20	40	LysoGb ₃ (t16:1) [LCB-3×H ₂ O+H] ⁺	
LysoGb ₃ (-2)	784.4	280.3	20	40	LysoGb ₃ (d18:2)[LCB-H ₂ O+H] ⁺	
LysoGb ₃ (+16)	802.4	280.3	20	40	LysoGb ₃ (t18:1) [LCB-2×H ₂ O+H] ⁺	
LysoGb ₃ (+18)	804.5	318.3	20	40	LysoGb ₃ (t18:0) [LCB+H] ⁺	
LysoGb ₃ (+34)	820.5	334.3	20	40	Not identified	Not identified
LysoGb ₃ (+50)	836.5	350.2	20	40	Not identified	Not identified

^aAdopted from Boutin M and Auray-Blais C, Anal. Chem. (2014) 86(7):3476-83.

IS, internal standard.

Supplementary Table 3 MRM table for LysoGb₃ species with C14-C26 LCB.

Category	Species	MS1	MS2				
		[M+H] ⁺	[LCB+H] ⁺	[LCB-H ₂ O+H] ⁺	[LCB-2×H ₂ O+H] ⁺	[LCB-3×H ₂ O+H] ⁺	[LCB-4×H ₂ O+H] ⁺
dX:0	d14:0	732.4	246.2	228.2	210.2	192.2	NA
	d15:0	746.4	260.2	242.2	224.2	206.2	NA
	d16:0	760.4	274.2	256.2	238.2	220.2	NA
	d17:0	774.4	288.2	270.2	252.2	234.2	NA
	d18:0	788.4	302.2	284.2	266.2	248.2	NA
	d19:0	802.4	316.2	298.2	280.2	262.2	NA
	d20:0	816.4	330.2	312.2	294.2	276.2	NA
	d21:0	830.4	344.2	326.2	308.2	290.2	NA
	d22:0	844.4	358.2	340.2	322.2	304.2	NA
	d23:0	858.4	372.2	354.2	336.2	318.2	NA
	d24:0	872.4	386.2	368.2	350.2	332.2	NA
	d25:0	886.4	400.2	382.2	364.2	346.2	NA
	d26:0	900.4	414.2	396.2	378.2	360.2	NA
dX:1	d14:1	730.4	244.2	226.2	208.2	190.2	NA
	d15:1	744.4	258.2	240.2	222.2	204.2	NA
	d16:1	758.4	272.2	254.2	236.2	218.2	NA
	d17:1	772.4	286.2	268.2	250.2	232.2	NA
	d18:1	786.4	300.2	282.2	264.2	246.2	NA
	d19:1	800.4	314.2	296.2	278.2	260.2	NA
	d20:1	814.4	328.2	310.2	292.2	274.2	NA
	d21:1	828.4	342.2	324.2	306.2	288.2	NA
	d22:1	842.4	356.2	338.2	320.2	302.2	NA
	d23:1	856.4	370.2	352.2	334.2	316.2	NA
	d24:1	870.4	384.2	366.2	348.2	330.2	NA
	d25:1	884.4	398.2	380.2	362.2	344.2	NA
	d26:1	898.4	412.2	394.2	376.2	358.2	NA
dX:2	d14:2	728.4	242.2	224.2	206.2	188.2	NA

	d15:2	742.4	256.2	238.2	220.2	202.2	NA
	d16:2	756.4	270.2	252.2	234.2	216.2	NA
	d17:2	770.4	284.2	266.2	248.2	230.2	NA
	d18:2	784.4	298.2	280.2	262.2	244.2	NA
	d19:2	798.4	312.2	294.2	276.2	258.2	NA
	d20:2	812.4	326.2	308.2	290.2	272.2	NA
	d21:2	826.4	340.2	322.2	304.2	286.2	NA
	d22:2	840.4	354.2	336.2	318.2	300.2	NA
	d23:2	854.4	368.2	350.2	332.2	314.2	NA
	d24:2	868.4	382.2	364.2	346.2	328.2	NA
	d25:2	882.4	396.2	378.2	360.2	342.2	NA
	d26:2	896.4	410.2	392.2	374.2	356.2	NA
dX:3	d14:3	726.4	240.2	222.2	204.2	186.2	NA
	d15:3	740.4	254.2	236.2	218.2	200.2	NA
	d16:3	754.4	268.2	250.2	232.2	214.2	NA
	d17:3	768.4	282.2	264.2	246.2	228.2	NA
	d18:3	782.4	296.2	278.2	260.2	242.2	NA
	d19:3	796.4	310.2	292.2	274.2	256.2	NA
	d20:3	810.4	324.2	306.2	288.2	270.2	NA
	d21:3	824.4	338.2	320.2	302.2	284.2	NA
	d22:3	838.4	352.2	334.2	316.2	298.2	NA
	d23:3	852.4	366.2	348.2	330.2	312.2	NA
	d24:3	866.4	380.2	362.2	344.2	326.2	NA
	d25:3	880.4	394.2	376.2	358.2	340.2	NA
	d26:3	894.4	408.2	390.2	372.2	354.2	NA
tX:0	t14:0	748.4	262.2	244.2	226.2	208.2	190.2
	t15:0	762.4	276.2	258.2	240.2	222.2	204.2
	t16:0	776.4	290.2	272.2	254.2	236.2	218.2
	t17:0	790.4	304.2	286.2	268.2	250.2	232.2

	t18:0	804.4	318.2	300.2	282.2	264.2	246.2
	t19:0	818.4	332.2	314.2	296.2	278.2	260.2
	t20:0	832.4	346.2	328.2	310.2	292.2	274.2
	t21:0	846.4	360.2	342.2	324.2	306.2	288.2
	t22:0	860.4	374.2	356.2	338.2	320.2	302.2
	t23:0	874.4	388.2	370.2	352.2	334.2	316.2
	t24:0	888.4	402.2	384.2	366.2	348.2	330.2
	t25:0	902.4	416.2	398.2	380.2	362.2	344.2
	t26:0	916.4	430.2	412.2	394.2	376.2	358.2
tX:1	t14:1	746.4	260.2	242.2	224.2	206.2	188.2
	t15:1	760.4	274.2	256.2	238.2	220.2	202.2
	t16:1	774.4	288.2	270.2	252.2	234.2	216.2
	t17:1	788.4	302.2	284.2	266.2	248.2	230.2
	t18:1	802.4	316.2	298.2	280.2	262.2	244.2
	t19:1	816.4	330.2	312.2	294.2	276.2	258.2
	t20:1	830.4	344.2	326.2	308.2	290.2	272.2
	t21:1	844.4	358.2	340.2	322.2	304.2	286.2
	t22:1	858.4	372.2	354.2	336.2	318.2	300.2
	t23:1	872.4	386.2	368.2	350.2	332.2	314.2
	t24:1	886.4	400.2	382.2	364.2	346.2	328.2
	t25:1	900.4	414.2	396.2	378.2	360.2	342.2
	t26:1	914.4	428.2	410.2	392.2	374.2	356.2

d, dihydroxy; t, trihydroxy; NA, not applicable; X, carbon number.

Supplementary Table 4 Instrument parameters for the quantification of LysoGb₃ and other compounds in this study using reversed-phase chromatography.

Instrument	Xevo TQ-S micro (Waters)
MS parameter	
Interface	ESI
Polarity	Positive
Capillary voltage	2.5 kV
Source temperature	150°C
Desolvation temperature	450°C
Flow rate of cone gas	50 L/h
Flow rate of desolvation gas	800 L/h
Analyzing mode	Multiple reaction monitoring
Dwell time	0.05 s
Data format	Centroid
UPLC parameter	
Column	UPLC BEH C18 (50 mm) Internal diameter: 2.1 µm Particle diameter: 1.7 µm
Column temperature	40°C
Weak wash solvent	0.2% formic acid in water/acetonitrile (50/50)
Strong wash solvent	0.2% formic acid in methanol
Mobile phase A	0.2% formic acid in water/acetonitrile (95/5)
Mobile phase B	0.2% formic acid in acetonitrile
Gradient (%B)	
LysoGb ₃ separation	0.0 - 1.0 min: 0% 1.0 - 3.0 min: 0 - 35% 3.0 - 5.5 min: 35 - 50% 5.51 - 7.0 min: 90% 7.01 - 9.0 min: 0%
Ceramide separation	0.0 - 0.5 min: 90% 0.5 - 6.0 min: 90-100% 6.0 - 10 min: 100% 10.1 - 12 min: 90%
Flow rate	0.5 mL/min
Injection volume	5 µL
Injection mode	Partial loop-fill method
Autosampler temperature	10°C

ESI, electrospray ionization.

Supplementary Table 5 MRM table for ceramide and Gb₃

Compound name	N-acyl group	LCB	MS1	MS2	Cone (V)	Collision (V)
			[M+H] ⁺	[LCB-2×H ₂ O+H] ⁺		
Ceramide	C14:0	d18:1	511.0	264.2	20	20
	C15:0	d18:1	525.0	264.2	20	20
	C16:0	d18:1	539.0	264.2	20	20
	C17:0 (Internal standard)	d18:1	553.0	264.2	20	20
	C18:0	d18:1	567.0	264.2	20	20
	C19:0	d18:1	581.0	264.2	20	20
	C20:0	d18:1	595.0	264.2	20	20
	C21:0	d18:1	609.0	264.2	20	20
	C22:0	d18:1	623.0	264.2	20	20
	C23:0	d18:1	637.0	264.2	20	20
	C24:0	d18:1	651.0	264.2	20	20
	C25:0	d18:1	665.0	264.2	20	20
	C26:0	d18:1	679.0	264.2	20	20
Gb ₃	C16:0	d18:1	1025.0	264.2	20	20

Supplementary Table 6 MRM table for sphinganine and sphingosine.

Compound name	LCB	MS1 [M+H] ⁺	MS2 [LCB-H ₂ O+H] ⁺	Cone (V)	Collision (V)
Sphinganine	d18:0	298.2	280.2	20	10
Sphingosine	d18:1	300.2	282.2	20	10