Supporting Information

The Effects of Gut Microbiome on Carcinogenic DNA Damage

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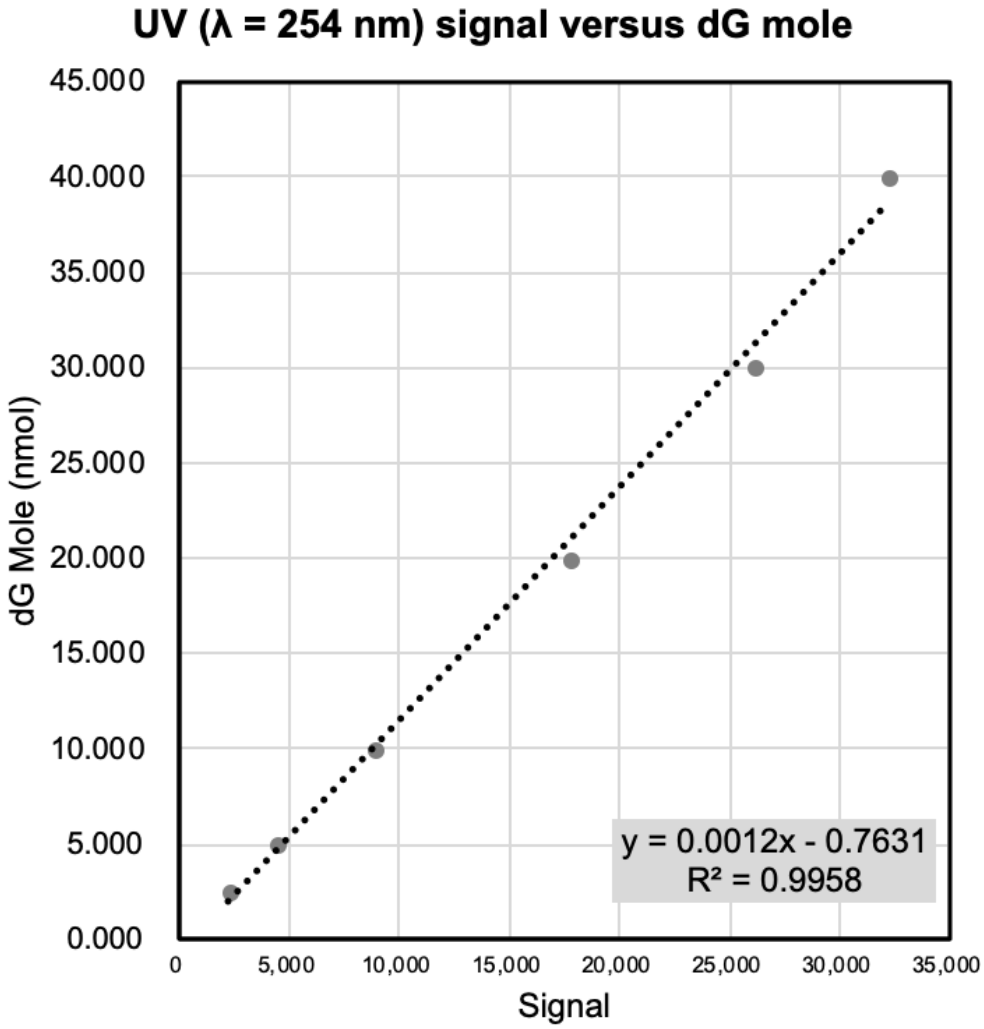

Figure S1. UV-signal to dG amount calibration curve on HPLC-UV system for dG quantification. The linear regression equation obtained (UV signal = 0.0012 × dG amount – 0.7631) can be used to measure dG amount in a sample.

Table S1 Sources and mutagenic properties for the 10 DNA adduct investigated in this study.

DNA Adduct	Source	Mutations	Reference
N^2-Me-dG	Methylating agent	G→A transition	[17, 26-28]
N^6-Me-dA	Methylating agent	AT→GC transition	[17, 26, 27]
N^2-Et-dG	Ethylating agent	G→C transversion	[17, 26, 27, 29]
OH-Me-dG	Formaldehyde	(No information for mutagenicity)[a]	[17, 27, 30, 31]
OH-Me-dA	Formaldehyde	(No information for mutagenicity)[a]	[17, 27, 30, 31]
N^2-EtD-dG	Acetaldehyde	(No information for mutagenicity)[a]	[17, 27, 30, 31]
O^6-Me-dG	Methylating agent	G→A transition	[17, 28]
1,N^2-ε-dG	Epoxides of enals from LPO	One base deletion (no complementary base)	[17, 32-36]
8-oxo-dG	Oxidative stress	GC→TA transition	[16, 17, 22, 23, 37]
5-Cl-dC	Neutrophil bactericidal activity forming HOCl	C→T transition	[38-40]

[a] Most research evaluates mutagenic properties of aldehyde-induced DNA adducts after $NaCNBH_3$ treatment to stabilize the primary DNA adduct structures. Consequently, the stable structures (N^2-Me-dG, N^6-Me-dA, N^2-Et-dG) are the adducts tested for mutagenic abilities. Thus, little investigation is done on the mutagenic properties of OH-Me-dG, OH-Me-dA, and N^2-EtD-dG.

Table S2. Linear regression equation to transfer the signal ratio between endogenous and internal standard adducts to their amount ratio for the 7 DNA adducts detected in this study. Intra- and inter-day accuracy and precision tests are also conducted and provided in the supplemental materials (Table S1).

DNA adduct	Linear Regression Equation[a]	R^2	Linear Range[b]
N^2-Me-dG	$y = 1.3775x + 0.0631$	0.9991	[0.0078,16]
N^6-Me-dA	$y = 0.8498x + 0.0128$	0.9997	[0.0078,16]
N^2-Et-dG	$y = 0.9810x + 0.0563$	0.9985	[0.0098,10]
O^6-Me-dG	$y = 0.5150x + 0.0472$	0.9989	[0.0078,16]
N^2- ε -dG	$y = 0.2329x + 0.0837$	0.9933	[0.0313,16]
8-oxo-dG	$y = 0.6486x + 1.3128$	0.9989	[0.0313,256]
5-Cl-dC	$y = 0.6649x + 0.0268$	0.9933	[0.0260,6.66]

[a] The x and the y in the linear regression equation represents the signal ratio and the amount ratio between the analytical standard and the internal standard of each DNA adducts, respectively.

[b] The available range for DNA adduct quantitation can be derived by multiplying the linear range of the amount ratio linear range to the spiked in internal standard in samples. As for N^2-Me-dG, N^6-Me-dA, N^2-Et-dG, O^6-Me-dG, N^2-ε-dG, and 8-oxo-dG, 2.5 fmol internal standard adducts were added individually. In addition, 1.5 fmol 5-Cl-dC internal standard is added to samples.

Table S3. Intra- and inter-day accuracy and precision evaluation for the 7 DNA adducts (analytical standards, AS) by spikes of each DNA adduct's internal standard. Determined by LC-MS/MS (n=3)

DNA Adduct	Linear Regression Equation [a]	R^2	Day	Added AS/IS Amount Ratio	Measured AS/IS Amount Ratio	RSD (%)	Accuracy (%)
N^2-Me-dG	$y = 1.3775x + 0.0631$	0.9991	Day 1	0.5	0.5016	4.77	96.22
				2	1.850	4.55	92.50
			Day 2	0.5	0.5355	4.03	92.91
				2	1.971	10.22	92.21
			Day 3	0.5	0.5060	2.09	98.45
				2	1.899	5.54	94.59
			Interday	0.5	0.5144	4.52	95.86
				2	1.907	6.93	93.10
N^6-Me-dA	$y = 0.8498x + 0.0128$	0.9997	Day 1	0.5	0.4612	3.80	92.24
				2	1.961	5.06	96.82
			Day 2	0.5	0.4673	2.50	93.46
				2	1.818	1.16	90.92
			Day 3	0.5	0.5368	3.39	92.65
				2	1.982	4.85	96.37

			Interday	0.5	0.4884	7.97	92.78
				2	1.920	5.42	94.70
N^2-Et-dG	$y = 0.9810x + 0.0563$	0.9985	Day 1	1.25	1.239	2.31	98.48
				5	4.785	3.47	95.71
			Day 2	1.25	1.263	3.96	96.56
				5	4.811	1.86	96.24
			Day 3	1.25	1.202	0.98	96.18
				5	4.724	3.84	94.49
			Interday	1.25	1.234	3.22	97.07
				5	4.773	2.86	95.48
O^6-Me-dG	$y = 0.5150x + 0.0472$	0.9989	Day 1	0.5	0.4525	7.62	90.50
				2	1.881	1.98	94.08
			Day 2	0.5	0.4980	2.85	98.11
				2	1.895	2.94	94.77
			Day 3	0.5	0.4316	6.03	86.31
				2	1.844	6.37	92.21
			Interday	0.5	0.4606	8.07	91.64
				2	1.873	3.81	93.69
		0.9933	Day 1	0.5	0.6129	4.81	77.41

1,N^2- ε -dG	$y = 0.8265x + 0.2917$		Day 2	2	1.674	3.91	83.73
				0.5	0.4501	3.86	90.01
				2	1.731	3.29	86.58
			Day 3	0.5	0.4538	7.44	90.75
				2	1.654	7.77	82.74
			Interday	0.5	0.5055	16.62	86.06
				2	1.686	5.03	84.35
8-oxo-dG	$y = 0.6486x + 1.3128$	0.9984	Day 1	8	8.310	0.43	96.12
				128	123.69	5.07	96.64
			Day 2	8	8.183	0.66	97.70
				128	128.98	5.71	95.83
			Day 3	8	8.040	0.40	99.49
				128	124.51	4.54	96.35
			Interday	8	8.178	1.50	97.77
				128	125.73	4.87	96.27
5-Cl-dC	$y = 0.6649x + 0.0268$	0.9985	Day 1	0.833	0.7762	2.20	93.14
				3.333	2.774	15.77	83.22
			Day 2	0.833	0.8137	12.24	90.05
				3.333	2.895	3.22	86.87

	Day 3	0.833	0.7056	13.29	84.67
		3.333	2.730	1.72	81.92
	Interday	0.833	0.7651	10.94	89.29
		3.333	2.800	8.46	84.00

[a] Both inter-day and intra-day calibration curve accuracy and precision test were conducted in 3 continuous days (See Supplemental Materials Table S3). The calibration curve of each DNA adducts conducted in the first day is listed in this table.

Table S4. Intra- and inter-day calibration curve tests for the 7 DNA adducts (analytical standards, AS) by spikes of each DNA adduct's internal standard. Determined by LC-MS/MS (n=3)

DNA Adduct	Day	Linear Regression Equation	R^2	Range
N^2-Me-dG	1	$y = 1.3775x + 0.0631$	0.9991	[0.0078,16]
	2	$y = 1.3278x + 0.1628$	0.9965	
	3	$y = 1.2913x + 0.1403$	0.9969	
N^6-Me-dA	1	$y = 0.8498x + 0.0128$	0.9997	[0.0078,16]
	2	$y = 0.8235x + 0.0646$	0.9995	
	3	$y = 0.7319x + 0.1674$	0.9950	
N^2-Et-dG	1	$y = 0.9810x + 0.0563$	0.9985	[0.0098,10]
	2	$y = 1.0118x + 0.0034$	0.9998	
	3	$y = 1.0585x - 0.0190$	0.9994	
O^6-Me-dG	1	$y = 0.5150x + 0.0472$	0.9989	[0.0078,16]
	2	$y = 0.5307x - 0.1103$	0.9969	
	3	$y = 0.5703x - 0.0107$	0.9996	
$1,N^2$- ε -dG	1	$y = 0.8265x + 0.2917$	0.9933	[0.0313,16]
	2	$y = 1.1322x + 0.0465$	0.9925	

	3	$y = 1.1005x + 0.0608$	0.9997	
8-oxo-dG	1	$y = 0.6486x + 1.328$	0.9984	[0.0313,256]
	2	$y = 0.6662x + 1.2519$	0.9975	
	3	$y = 0.6580x + 1.0978$	0.9984	
5-Cl-dC	1	$y = 0.5846x + 0.1168$	0.9981	[0.0260,6.66]
	2	$y = 0.6449x + 0.0268$	0.9985	
	3	$y = 0.5438x + 0.0968$	0.9936	

Table S5. Descriptive statistics for dG amount and N^2-Me-dG adduct quantification in liver of GF (n=5) and CONV-R (n=5) mice.

GF/ CONV-R	Mice subject	dG amount (nmol)[a]	Endogenous/IS N^2-Me-dG signal ratio[b]	Endogenous N^2-Me-dG amount (nmol)[c]	Normalized N^2-Me-dG amount (adducts/10^8 dG)[d]	Mean±SEM (adducts/10^8 dG)[e]
GF	#1	26.13	0.5238	1.9617	7.506	7.4186±0.1595
	#2	22.98	0.4867	1.8339	7.980	
	#3	27.59	0.5185	1.9432	7.044	
	#4	27.31	0.5368	2.0065	7.346	
	#5	27.22	0.5246	1.9643	7.217	
CONV-R	#1	27.24	0.5522	2.0592	7.559	7.0913±0.5675
	#2	26.08	0.6102	2.2590	8.661	
	#3	26.74	0.3548	1.3795	5.158	
	#4	26.58	0.5048	1.8960	7.134	
	#5	26.89	0.4965	1.8675	6.945	

[a] The dG signal for each digested DNA sample is detected during the DNA adduct enrichment with a HPLC-UV system. dG is detected by absorbance at λ = 254 nm. A calibration curve between dG amount (nmol) and dG signal under UV detection (λ = 254 nm) was prepared before analysis and was used for dG quantification.

[b] Endogenous and IS N^2-Me-dG was measured on a LC-MS/MS system by PRM mode. The signal of quantifying product ions chosen for the endogenous and IS N^2-Me-dG was recorded and used to obtain the endogenous/IS N^2-Me-dG signal ratio.

[c] The endogenous/IS N^2-Me-dG amount ratio was obtained by fitting the endogenous/IS N^2-Me-dG signal ratio into a prepared calibration curve (Table S2.). For every sample presented in this study, 2.5 fmol of IS for N^2-Me-dG was added prior to DNA digestion for quantifying N^2-Me-dG. By multiplying this amount, absolute quantification of the endogenous N^2-Me-dG can be obtained (in nmol).

[d] The amount of dG was used to normalize the amount of endogenous N^2-Me-dG. The normalized N^2-Me-dG amount was presented in the number of N^2-Me-dG per 10^8 dG.

[e] Mean values of N^2-Me-dG quantities are presented with their standard error of mean (SEM).