

**Supplementary Table S1.** Summary of general information of metabolites in serum and urine in experimental

	Healthy	Subclinical ketosis
Serum		
Detected metabolites (n≥1)	98	83
Quantified metabolites (n=3)	52	55
Classified chemical class	12	12
Range of metabolites concentration	2.20 ~ 856.50 μM	0.90 ~ 686.70 μM
Urine		
Detected metabolites (n≥1)	144	168
Quantified metabolites (n=3)	93	93
Classified chemical class	13	14
Range of metabolites concentration	1.60 ~ 28,538.30 μM	1.10 ~ 31,683.70 μM

**Supplementary Table S2.** Quantified metabolites concentration (alcohols, aliphatic acylic compounds, amino acids, benzoic acids and carbohydrates classes) in serum of healthy and subclinical ketosis group by proton nuclear magnetic resonance spectroscopy analysis

Metabolites (μM)	Healthy	Subclinical ketosis
Alcohols		
Methanol	23.23 ± 2.32 (9.99)	23.75 ± 1.06 (4.46)
Aliphatic acylic compounds		
Trimethylamine <i>N</i> -oxide	5.23 ± 2.64 (50.48)	7.43 ± 5.53 (74.43)
Amino acids		
Alanine	53.40 ± 4.26 (7.98)	38.65 ± 18.46 (47.76)
Anserine	10.20 ± 1.21 (11.86)	5.50 ± 3.31 (60.18)
Arginine	39.00 ± 31.54 (80.87)	NQ
Creatine	42.90 ± 13.09 (30.51)	30.00 ± 24.49 (81.63)
Glycine	89.87 ± 3.09 (3.44)	92.87 ± 29.73 (32.01)
Isoleucine	15.03 ± 9.65 (64.20)	15.95 ± 4.74 (29.72)
Leucine	19.80 ± 12.87 (65.00)	16.27 ± 10.43 (64.11)
Methionine	NQ	1.80 ± 0.42 (23.33)
<i>N</i> -isovaleroylglycine	3.75 ± 1.63 (43.47)	2.95 ± 1.06 (35.93)
<i>N</i> -phenylacetyl glycine	6.87 ± 3.13 (45.56)	6.80 ± 3.96 (58.24)
Valine	12.43 ± 10.52 (84.63)	11.80 ± 0.17 (1.44)
Benzoic acids		
4-hydroxy-3-methoxymandelate	NQ	2.25 ± 0.49 (21.78)
Homogentisate	NQ	2.90 ± 0.00
Syringate	1.17 ± 0.65 (55.56)	NQ
Carbohydrates		
Acetoacetate	4.37 ± 2.83 (64.76)	13.80 ± 1.42 (10.29)
Erythritol	9.03 ± 0.65 (7.20)	NQ
Fructose	NQ	13.45 ± 1.77 (13.16)
Galactose	NQ	23.10 ± 0.14 (0.61)
Glucose	273.23 ± 62.15 (22.75)	254.00 ± 29.82 (11.74)
Glucose-6-phosphate	NQ	24.55 ± 9.97 (40.61)
Glutathione	NQ	11.85 ± 1.48 (12.49)
Lactulose	19.10 ± 4.51 (23.61)	12.87 ± 2.41 (18.73)
Mannose	15.20 ± 1.98 (13.03)	NQ
Pyruvate	14.15 ± 7.71 (54.49)	29.37 ± 8.87 (30.20)
Ribose	NQ	79.55 ± 16.90 (21.24)
Succinate	4.13 ± 2.03 (49.15)	10.93 ± 2.80 (25.62)
Sucrose	4.35 ± 0.21 (4.83)	NQ

Mean ± standard deviation (% of coefficient of variation), n=3. NQ, Not quantified.

**Supplementary Table S3.** Quantified metabolites concentration (carboxylic acids, indoles and lipids classes) in serum of healthy and subclinical ketosis group by proton nuclear magnetic resonance spectroscopy analysis

Metabolites (μM)	Healthy	Subclinical ketosis
<b>Carboxylic acids</b>		
2-hydroxyisobutyrate	1.05 ± 0.35 (33.33)	NQ
3-hydroxyisovalerate	15.23 ± 0.25 (1.64)	9.53 ± 7.94 (83.32)
5-aminolevulinate	9.10 ± 0.96 (10.55)	3.37 ± 1.10 (32.64)
Creatine phosphate	8.03 ± 5.34 (66.50)	3.70 ± 2.40 (64.86)
Glycylproline	13.80 ± 2.16 (15.65)	NQ
Guanidoacetate	9.35 ± 3.04 (32.51)	5.30 ± 4.38 (82.64)
Hydroxyacetone	3.20 ± 0.71 (22.19)	NQ
Malonate	6.90 ± 2.41 (34.93)	8.93 ± 2.58 (28.89)
Pantothenate	3.05 ± 0.49 (16.07)	3.77 ± 1.18 (31.30)
<b>Indoles</b>		
5-hydroxyindole-3-acetate	NQ	3.50 ± 0.42 (12.00)
<b>Lipids</b>		
2-hydroxyisovalerate	57.17 ± 13.45 (23.53)	53.23 ± 10.75 (20.20)
3-hydroxy-3-methylglutarate	NQ	14.10 ± 15.70 (111.35)
3-hydroxybutyrate	142.20 ± 62.47 (43.93)	157.83 ± 47.69 (30.22)
3-Methylglutarate	23.97 ± 13.53 (56.45)	22.00 ± 4.37 (19.86)
Carnitine	2.10 ± 0.28 (13.33)	9.40 ± 3.82 (40.64)
Glutaric acid monomethyl ester	10.60 ± 1.84 (17.36)	8.30 ± 2.69 (32.41)
Thymol	NQ	2.30 ± 0.14 (6.09)

Mean ± standard deviation (% of coefficient of variation), n=3 NQ, Not quantified.

**Supplementary Table S4.** Quantified metabolites concentration (organic acids and others classes) in serum of healthy and subclinical ketosis group by proton nuclear magnetic resonance spectroscopy analysis

Metabolites (μM)	Healthy	Subclinical ketosis
<b>Organics acids</b>		
Acetate	271.60 ± 54.60 (20.10)	343.93 ± 45.40 (13.20)
Formate	12.87 ± 1.19 (9.25)	15.40 ± 0.42 (2.73)
Gluconate	42.87 ± 25.70 (59.95)	49.60 ± 3.25 (6.55)
Lactate	610.73 ± 213.00 (34.88)	565.50 ± 106.69 (18.87)
Malate	48.40 ± 14.57 (30.10)	49.13 ± 5.71 (11.62)
<i>N</i> -nitrosodimethylamine	NQ	5.85 ± 4.45 (76.07)
O-acetylcholine	NQ	5.70 ± 1.84 (32.28)
Phenylacetate	10.80 ± 2.69 (24.91)	9.00 ± 1.30 (14.44)
<b>Others</b>		
1,7-dimethylxanthine	4.35 ± 1.34 (30.80)	5.35 ± 0.64 (11.96)
3-methylxanthine	2.00 ± 0.56 (28.00)	NQ
Acetone	34.97 ± 31.93 (91.31)	10.97 ± 1.77 (16.13)
Arabinose	42.40 ± 2.55 (6.01)	NQ
Betaine	15.03 ± 2.14 (14.24)	5.97 ± 2.64 (44.22)
Galactarate	12.00 ± 4.95 (41.25)	11.57 ± 1.69 (14.61)
Ibuprofen	5.05 ± 4.03 (79.80)	6.95 ± 0.21 (3.02)
Levulinate	14.65 ± 0.35 (2.39)	9.10 ± 5.76 (63.30)
Melatonin	3.15 ± 0.78 (24.76)	3.35 ± 0.49 (14.63)
Pyridoxine	1.55 ± 0.07 (4.52)	2.05 ± 0.07 (3.41)
sn-glycero-3-phosphocholine	10.87 ± 4.36 (40.11)	14.00 ± 6.65 (47.50)

Mean ± standard deviation (% of coefficient of variation), n=3.

NQ, Not quantified.

**Supplementary Table S5.** Quantified metabolites concentration (aliphatic acylic compounds, amines, amino acids, benzoic acids and carbohydrates classes) in urine of healthy and subclinical ketosis group by proton nuclear magnetic resonance spectroscopy analysis

Metabolites ( $\mu\text{M}$ )	Healthy	Subclinical ketosis
Aliphatic acylic compounds		
O-phosphocholine	$1.60 \pm 1.10$ (68.75)	$1.45 \pm 0.49$ (33.79)
Trimethylamine <i>N</i> -oxide	$1,036.80 \pm 104.20$ (10.50)	$2,023.93 \pm 1,527.23$ (75.46)
Urea	$28,538.30 \pm 5036.50$ (17.65)	$22,579.30 \pm 8,394.82$ (37.18)
Amines		
Carnosine	$19.30 \pm 12.30$ (63.73)	$32.93 \pm 11.37$ (34.53)
Dimethylamine	$70.20 \pm 30.47$ (43.40)	$76.63 \pm 43.75$ (57.09)
Histamine	$23.25 \pm 9.25$ (39.78)	$18.00 \pm 9.56$ (53.11)
Sarcosine	$16.85 \pm 6.15$ (36.50)	$18.65 \pm 8.98$ (48.15)
Amino acids		
2-furoylglycine	$14.15 \pm 4.75$ (33.57)	$20.30 \pm 13.06$ (64.33)
Anserine	$39.47 \pm 45.86$ (116.19)	$14.15 \pm 6.25$ (44.17)
Creatine	$950.10 \pm 462.60$ (48.69)	$1,475.93 \pm 1,719.71$ (116.52)
Histidine	$22.60 \pm 14.20$ (62.83)	$41.40 \pm 18.99$ (45.87)
<i>N</i> -phenylacetylglutamine	$1,038.85 \pm 545.25$ (52.49)	$1,180.30 \pm 790.55$ (66.98)
$\pi$ -methylhistidine	$18.40 \pm 5.37$ (29.18)	$6.00 \pm 0.00$
Alanine	$59.80 \pm 31.28$ (52.31)	$100.70 \pm 27.90$ (27.71)
Glycine	$122.35 \pm 110.52$ (90.33)	$33.10 \pm 0.00$
Hippurate	$9,254.55 \pm 1,835.05$ (19.83)	$6,851.37 \pm 1,548.99$ (22.61)
Tryptophan	$226.30 \pm 29.70$ (13.12)	$171.25 \pm 55.08$ (32.16)
Xanthurenate	$34.00 \pm 2.30$ (6.76)	$42.30 \pm 55.01$ (130.05)
Benzoic acids		
3-hydroxymandelate	$5.55 \pm 1.05$ (18.92)	$28.47 \pm 21.43$ (75.27)
4-hydroxy-3-methoxymandelate	$16.15 \pm 8.85$ (54.80)	$18.45 \pm 12.09$ (65.53)
4-hydroxyphenylacetate	$94.70 \pm 13.00$ (13.73)	$150.60 \pm 50.91$ (33.80)
5-methoxysalicylate	$88.70 \pm 45.36$ (51.14)	$33.40 \pm 29.33$ (87.81)
Acetylsalicylate	$100.60 \pm 18.50$ (18.39)	$134.57 \pm 175.06$ (130.09)
Gentisate	$50.20 \pm 44.16$ (87.97)	$203.97 \pm 120.28$ (58.97)
Mandelate	$62.05 \pm 7.65$ (12.33)	$54.60 \pm 35.41$ (64.85)
Syringate	$318.70 \pm 266.10$ (83.50)	$120.57 \pm 143.29$ (118.84)
Vanillate	$184.85 \pm 87.85$ (47.53)	$91.70 \pm 54.39$ (59.31)
o-cresol	$85.20 \pm 40.20$ (47.18)	$106.80 \pm 91.08$ (85.28)
p-cresol	$33.50 \pm 1.40$ (4.18)	$54.67 \pm 28.16$ (51.51)
Carbohydrates		
Acetoacetate	$166.90 \pm 12.11$ (7.26)	$256.43 \pm 166.92$ (65.09)
Fructose	$306.85 \pm 161.25$ (52.55)	$152.60 \pm 64.47$ (42.25)
Galactitol	$190.30 \pm 163.20$ (85.76)	$78.23 \pm 49.74$ (63.58)
Glucuronate	$71.95 \pm 8.45$ (11.74)	$90.55 \pm 21.71$ (23.98)
Lactose	$1,674.20 \pm 982.80$ (58.70)	$1,043.43 \pm 786.56$ (75.38)
Succinate	$15.90 \pm 11.78$ (74.09)	$33.83 \pm 15.67$ (46.32)

Mean  $\pm$  standard deviation (% of coefficient of variation), n=3

**Supplementary Table S6.** Quantified metabolites concentration (carboxylic acids, imidazolinones, indoles and lipids classes) in urine of healthy and subclinical ketosis group by proton nuclear magnetic resonance spectroscopy analysis

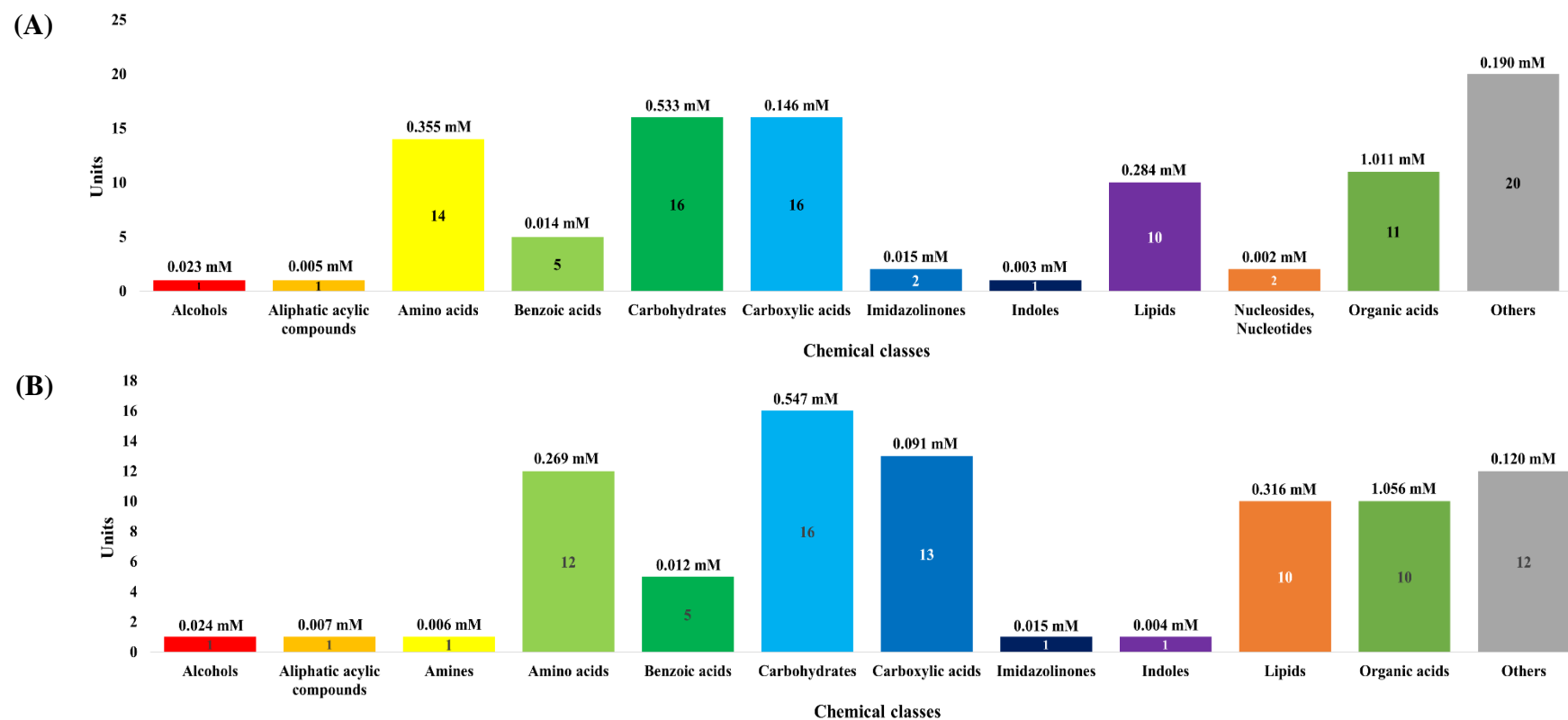
Metabolites (μM)	Healthy	Subclinical ketosis
<b>Carboxylic acids</b>		
3-hydroxyisovalerate	23.10 ± 7.60 (32.90)	21.30 ± 11.36 (53.33)
3-hydroxyphenylacetate	73.75 ± 18.65 (25.29)	92.17 ± 43.67 (47.38)
5-aminolevulinate	56.30 ± 21.98 (39.04)	15.07 ± 13.08 (86.79)
Creatine phosphate	199.00 ± 191.70 (96.33)	554.83 ± 887.45 (159.95)
Glycylproline	584.10 ± 147.56 (25.26)	354.05 ± 82.52 (23.31)
Guanidoacetate	52.60 ± 29.33 (55.76)	47.93 ± 25.70 (53.62)
Homocystine	130.30 ± 67.00 (51.42)	236.55 ± 63.29 (26.76)
Homovanillate	29.70 ± 11.88 (40.00)	24.05 ± 12.80 (53.22)
Malonate	35.35 ± 18.95 (53.61)	160.97 ± 242.29 (150.52)
<i>N</i> -acetyltyrosine	58.40 ± 65.25 (111.73)	60.67 ± 70.35 (115.96)
<i>N</i> 6-acetyllysine	54.75 ± 11.75 (21.46)	44.80 ± 7.50 (16.74)
Pantothenate	73.65 ± 29.45 (39.99)	48.57 ± 36.72 (75.60)
trans-aconitate	12.75 ± 3.65 (28.63)	12.00 ± 13.86 (115.50)
<b>Imidazolinones</b>		
Allantoin	2,668.40 ± 2,616.50 (98.06)	2,672.80 ± 1,177.81 (44.07)
Creatinine	1,024.40 ± 888.54 (86.74)	447.35 ± 583.86 (130.52)
Imidazole	16.70 ± 7.34 (43.95)	10.60 ± 3.80 (35.85)
<b>Indoles</b>		
3-indoxylsulfate	149.45 ± 60.35 (40.38)	101.07 ± 13.64 (13.50)
5-hydroxyindole-3-acetate	83.60 ± 0.60 (0.72)	69.57 ± 36.51 (52.48)
<b>Lipids</b>		
2-methylglutarate	71.10 ± 45.40 (63.85)	104.30 ± 41.72 (40.00)
3-methylglutarate	206.90 ± 45.97 (22.22)	199.10 ± 76.45 (38.40)
Carnitine	7.95 ± 2.95 (37.11)	7.70 ± 3.54 (45.97)
Choline	1.60 ± 0.79 (49.38)	1.85 ± 0.49 (26.49)
Glutaric acid monomethyl ester	9.60 ± 15.44 (160.83)	21.07 ± 16.64 (78.97)
Glycocholate	67.60 ± 40.70 (60.21)	15.15 ± 10.68 (70.50)
Glycolate	8,743.25 ± 725.05 (8.29)	3,987.80 ± 5,988.27 (150.16)
Methylsuccinate	127.95 ± 22.65 (17.70)	95.65 ± 111.79 (116.87)
Sebacate	241.40 ± 105.37 (43.65)	219.60 ± 114.33 (52.06)
Thymol	56.50 ± 5.80 (10.27)	33.15 ± 9.97 (30.08)

Mean ± standard deviation (% of coefficient of variation), n=3

**Supplementary Table S7.** Quantified metabolites concentration (nucleosides & nucleotides, organic acids and others classes) in urine of healthy and subclinical ketosis group by proton nuclear magnetic resonance spectroscopy analysis

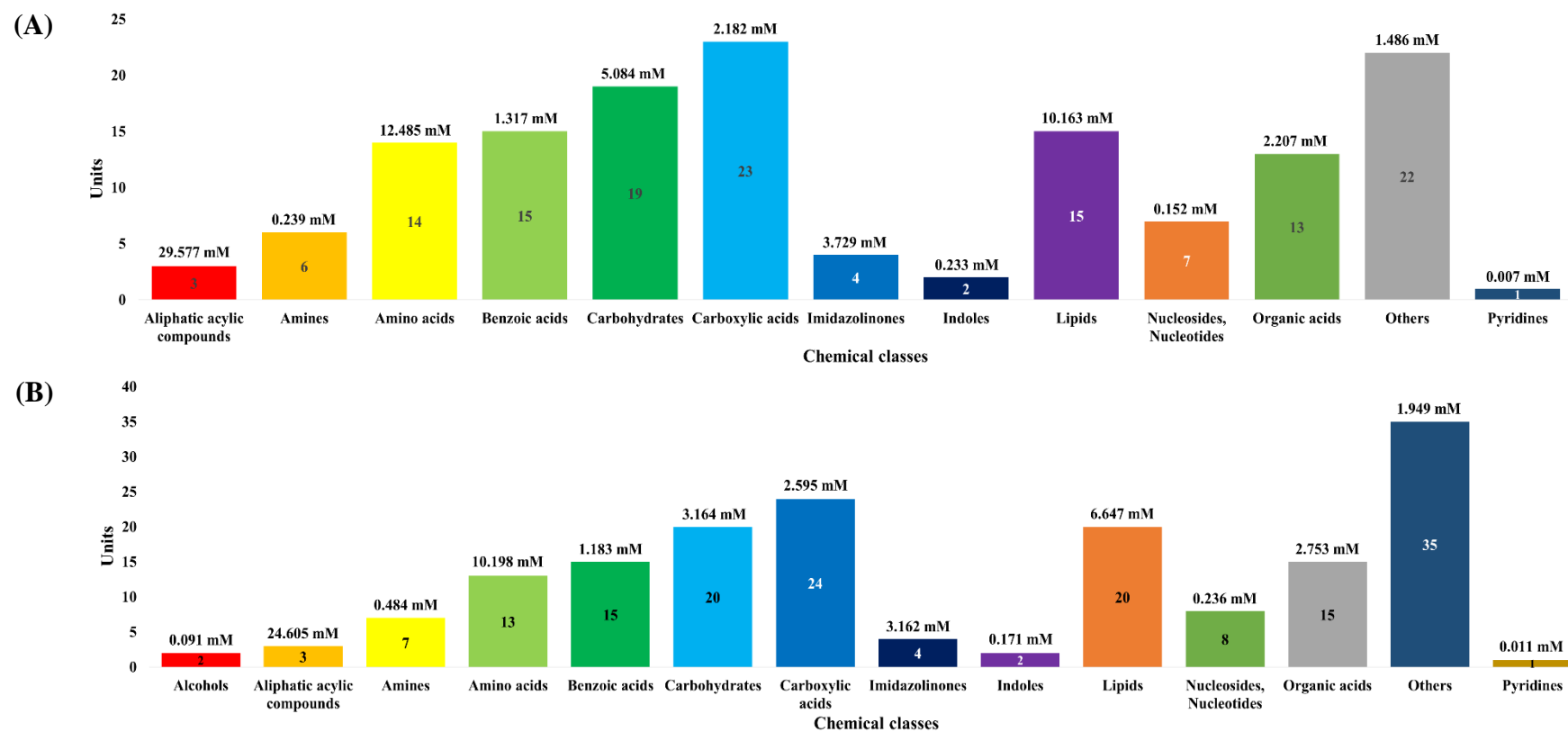
Metabolites ( $\mu\text{M}$ )	Healthy	Subclinical ketosis
Nucleosides & Nucleotides		
Xanthine	$116.40 \pm 8.00$ (6.87)	$146.00 \pm 43.02$ (29.47)
dTTP	$3.40 \pm 3.57$ (105.00)	$22.60 \pm 24.47$ (108.27)
Organic acids		
3-hydroxykynurenine	$95.60 \pm 55.34$ (57.89)	$128.03 \pm 78.39$ (61.23)
Acetate	$795.10 \pm 168.45$ (21.19)	$1,466.63 \pm 706.94$ (48.20)
Formate	$102.15 \pm 15.05$ (14.73)	$111.75 \pm 35.28$ (31.57)
Fumarate	$3.30 \pm 2.11$ (63.94)	$2.75 \pm 1.34$ (48.73)
Malate	$296.80 \pm 96.90$ (32.65)	$194.17 \pm 53.73$ (27.67)
<i>N</i> -nitrosodimethylamine	$28.60 \pm 0.00$	$92.73 \pm 25.24$ (27.22)
Nicotinurate	$7.00 \pm 3.78$ (54.00)	$10.77 \pm 2.59$ (24.05)
Phenylacetate	$147.15 \pm 110.85$ (75.33)	$47.70 \pm 27.73$ (58.13)
Salicylate	$90.55 \pm 29.75$ (32.85)	$329.60 \pm 387.35$ (117.52)
Succinylacetone	$115.65 \pm 57.75$ (49.94)	$65.13 \pm 16.85$ (25.87)
Others		
2-hydroxyphenylacetate	$45.10 \pm 15.44$ (34.24)	$30.85 \pm 13.51$ (43.79)
3-phenylpropionate	$118.95 \pm 95.85$ (80.58)	$208.20 \pm 175.67$ (84.38)
4-pyridoxate	$16.45 \pm 2.15$ (13.07)	$11.50 \pm 5.01$ (43.57)
Acetone	$19.10 \pm 11.10$ (58.12)	$124.30 \pm 157.54$ (126.74)
Betaine	$67.75 \pm 58.75$ (86.72)	$10.60 \pm 5.90$ (55.66)
Biotin	$219.95 \pm 130.05$ (59.13)	$87.30 \pm 42.86$ (49.10)
Caffeine	$17.90 \pm 6.40$ (35.75)	$8.83 \pm 8.12$ (91.96)
Cellobiose	$143.15 \pm 61.25$ (42.79)	$48.30 \pm 24.51$ (50.75)
Desaminotyrosine	$203.10 \pm 56.11$ (27.63)	$125.60 \pm 17.96$ (14.30)
Epicatechin	$9.75 \pm 6.45$ (66.15)	$26.00 \pm 22.49$ (86.50)
Galactarate	$69.20 \pm 16.80$ (24.28)	$102.30 \pm 53.46$ (52.26)
Indole-3-acetate	$53.30 \pm 1.60$ (3.00)	$108.57 \pm 55.24$ (50.88)
Indole-3-lactate	$66.30 \pm 0.00$	$92.40 \pm 27.81$ (30.10)
Kynurenate	$8.25 \pm 5.05$ (61.21)	$11.10 \pm 10.75$ (96.85)
Melatonin	$100.70 \pm 51.70$ (51.34)	$40.63 \pm 18.30$ (45.04)
<i>N</i> -methylhydantoin	$54.10 \pm 45.11$ (83.38)	$6.45 \pm 6.15$ (95.35)
Riboflavin	$14.90 \pm 13.70$ (91.95)	$15.23 \pm 7.66$ (50.30)
$\tau$ -methylhistidine	$32.23 \pm 13.14$ (40.77)	$57.65 \pm 34.95$ (60.62)

Mean  $\pm$  standard deviation (% of coefficient of variation), n=3.



**Suppl Fig 1.** The classification of detected serum metabolites according to chemical classes in healthy (A) and subclinical ketosis (B) group by proton nuclear magnetic resonance spectroscopy analysis. Each square box color indicates the classification of metabolites, the number represents the detected metabolites, and the numbers in parentheses indicate the sum of the total concentration of the detected metabolites.





**Suppl Fig 2.** The classification of detected urine metabolites according to chemical classes in healthy (A) and subclinical ketosis (B) group by proton nuclear magnetic resonance spectroscopy analysis. Each square box color indicates the classification of metabolites, the number represents the detected metabolites, and the numbers in parentheses indicate the sum of the total concentration of the detected metabolites.

