Primary 12α-hydroxylated bile acids lower hepatic iron concentration in rats

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Online Supplementary Material



**Supplemental Figure 1. Study design.**

**Supplemental Table 1. Diet compositions in the present studies (Studies 1-3)**

 Control CA

 g/kg diet

Casein1 200 200

Dextrin2 529.5 529.5

Sucrose3 100 99.5

Soybean oil4 70 70

Cellulose5 50 50

Mineral mixture6 35  35

Vitamin mixture7 10 10

L-Cystine8 3 3

Choline hydrogen tartrate8 2.5 2.5

Cholic acid8 - 0.5

1 NZMP Acid Casein (Fonterra Co-Operative Group Limited, Auckland, New Zealand),

2 TK-16 (Matsutani Chemical Industry Co., Ltd., Hyogo, Japan)

3 Nippon Beet Sugar Manufacturing Co., Ltd., Tokyo, Japan

4 J-Oil Mills, Inc., Tokyo, Japan

5 Crystalline cellulose (Ceolus PH-102, Asahi Kasei Chemicals Corp., Tokyo, Japan)

6 AIN-93G Mineral mixture [(1)](https://paperpile.com/c/q9dbaa/nI5WO)

7 AIN-93G Mineral mixture [(1)](https://paperpile.com/c/q9dbaa/nI5WO)

8 FUJIFILM Wako Pure Chemical Industries, Ltd., Osaka, Japan

**Reference**

1. [Reeves PG, Nielsen FH, Fahey GC Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr. 1993;123:1939–51.](http://paperpile.com/b/q9dbaa/nI5WO)

**Supplemental Table 2. Jejunal content BA profile in WKAH/HkmSlc male rats fed either control or CA diet for 2 weeks (Study 1)**

 Control CA *P*-value

**Primary 12αOH BAs,** **µmol/g jejunal contents**

CA 8.4 ± 1.4 23 ± 4.6\* 0.021

TCA 4.6 ± 1.8 32 ± 10 \* < 0.001

GCA 0.06 ± 0.02 0.8 ± 0.3\* < 0.001

**Secondary 12αOH BAs, µmol/g jejunal contents**

DCA 0.1 ± 0.03 0.5 ± 0.2 0.067

TDCA 0.09 ± 0.03 5.4 ± 2.6\* 0.029

GDCA 0.02 ± 0.02 0.03 ± 0.01 0.10

7oDCA 0.5 ± 0.2 1.8 ± 0.5\* 0.020

12oLCA 0 0

3o12α 0.008 ± 0.002 0.007 ± 0.002 0.77

UCA 0.7 ± 0.2 1.3 ± 0.3 0.13

**Primary non-12αOH BAs, µmol/g jejunal contents**

CDCA 0.1 ± 0.02 0.07 ± 0.02 0.21

TCDCA 0.1 ± 0.05 0.2 ± 0.08 0.29

GCDCA 0.01 ± 0.003 0.01 ± 0.004 0.77

αMCA 0.2 ± 0.06 0.09 ± 0.03 0.21

βMCA 0.7 ± 0.07 0.6 ± 0.2 0.84

TαMCA 0.05 ± 0.04 0.04 ± 0.03 0.72

TβMCA 3.1 ± 1.2 6.7 ± 3.8 0.85

**Secondary non-12αOH BAs, µmol/g jejunal contents**

ωMCA 0.08 ± 0.04 0.05 ± 0.01 0.36

TωMCA 0.6 ± 0.1 0.5 ± 0.3 0.054

HCA 0 0

HDCA 0 0

THDCA 0.03 ± 0.01 1.3 ± 1.0\* < 0.001

GHDCA 0 0

UDCA 0.07 ± 0.01 0.07 ± 0.02 0.92

TUDCA 0.002 ± 0.002 0.2 ± 0.1 0.24

GUDCA 0.01 ± 0.01 0.01 ± 0.004 0.63

LCA 0.02 ± 0.01 0.001 ± 0.001 0.37

TLCA 0.07 ± 0.01 0.04 ± 0.01\* 0.034

GLCA 0 0

7oLCA 0.01 ± 0.003 0.01 ± 0.002 0.80

Values are shown as the mean ± SEM (n = 8 for control, n = 9 for CA group). The values of undetectable parameters were considered to be 0 in the statistical analysis. A significant difference in mean value between the two groups was determined using the unpaired Student *t*-test. In cases of violation of the assumptions of normality or homogeneity of variance, the Mann-Whitney U test or Welch *t*-test was applied, respectively. \*Significantly different from the control (*P* < 0.05).

**Supplemental Table 3. Portal plasma BA profile in WKAH/HkmSlc male rats fed either control or CA diet for 2 weeks (Study 1)**

 Control CA *P*-value

**Primary 12αOH BAs,** **µmol/L portal plasma**

CA 12 ± 2.7 48 ± 14\* 0.030

TCA 3.7 ± 1.6 77 ± 18\* < 0.001

GCA 0 1.5 ± 0.4\* < 0.001

**Secondary 12αOH BAs, µmol/L portal plasma**

DCA 0.6 ± 0.1 2.9 ± 0.7\* 0.006

TDCA 0 6.2 ± 1.6\* 0.003

GDCA 0 0.2 ± 0.06\* 0.027

7oDCA 0.8 ± 0.3 3.3 ± 0.8\* 0.014

12oLCA 0 0

3o12α 0.2 ± 0.04 0.2 ± 0.03 0.25

UCA 1.6 ± 0.4 3.6 ± 0.8\* 0.049

**Primary non-12αOH BAs, µmol/L portal plasma**

CDCA 0.6 ± 0.2 0.5 ± 0.1 0.60

TCDCA 0.3 ± 0.6 0.5 ± 0.1 0.27

GCDCA 0.1 ± 0.05 0.1 ± 0.06 0.63

αMCA 0.5 ± 0.2 0.6 ± 0.2 0.47

βMCA 2.5 ± 0.5 2.9 ± 0.8 0.66

TαMCA 0 4.0 ± 1.7\* 0.017

TβMCA 7.9 ± 3.2 1.4 ± 1.0\* 0.022

**Secondary non-12αOH BAs, µmol/L portal plasma**

ωMCA 2.3 ± 0.6 1.6 ± 0.4 0.44

TωMCA 0.8 ± 0.3 0.4 ± 0.1 0.27

HCA 0 0

HDCA 0 0

THDCA 0.08 ± 0.05 0.4 ± 0.1\* 0.037

GHDCA 0 0

UDCA 0.3 ± 0.06 0.3 ± 0.06 0.36

TUDCA 0.02 ± 0.02 0.5 ± 0.1\* 0.005

GUDCA 0.2 ± 0.07 0.1 ± 0.04 0.48

LCA 0.4 ± 0.1 0.6 ± 0.2 0.35

TLCA 0.03 ± 0.03 0.1 ± 0.05 0.30

GLCA 0 0

7oLCA 0.07 ± 0.04 0.1 ± 0.04 0.47

Values are shown as the mean ± SEM (n = 8 for control, n = 9 for CA group). The values of undetectable parameters were considered to be 0 in the statistical analysis. A significant difference in mean value between the two groups was determined using the unpaired Student *t*-test. In cases of violation of the assumptions of normality or homogeneity of variance, the Mann-Whitney U test or Welch *t*-test was applied, respectively. \*Significantly different from the control (*P* < 0.05).

**Supplemental Table 4.** **Food intake, growth, organ weights, and fecal iron excretion in WKAH/HkmSlc male rats fed either control or CA diet (Study 1)**

 Control CA *P* value

Total food intake, g/2 weeks 223 ± 6.8 208 ± 5.5 0.10

Final body weight, g 193 ± 4.4 191 ± 4.3 0.79

Liver weight, g 9.2 ± 0.3 9.4 ± 0.2 0.62

Epididymal adipose tissue, g 3.2 ± 0.2 2.8 ± 0.2 0.068

Fecal iron excretion, mg/day 0.73 ± 0.08 0.66 ± 0.07 0.79

Values are shown as the mean ± SEM (n = 8 for control, n = 9 for CA group). A significant difference in mean value between the two groups was determined using the unpaired Student *t*-test. In cases of violation of the assumptions of normality or homogeneity of variance, the Mann-Whitney U test or Welch *t*-test was applied, respectively.

**Supplemental Table 5.** **Food intake, growth, and organ weights** **in WKAH/HkmSlc male rats fed either control or CA diet for 13 weeks (Study 2)**

 Control CA  *P* value

Total food intake, g/13 weeks 1614 ± 26 1617 ± 24 0.94

Final body weight, g 387 ± 7.8 390 ± 5.4 0.75

Liver weight, g 12.0 ± 0.4 14.4 ± 0.3\* < 0.001

Epididymal adipose tissue, g 8.5 ± 0.3 8.1 ± 0.3 0.30

Values are shown as the mean ± SEM (n = 12). A significant difference in mean value between the two groups was determined using the unpaired Student *t*-test. In cases of violation of the assumptions of normality or homogeneity of variance, the Mann-Whitney U test or Welch *t*-test was applied, respectively. \*Significantly different from the control (*P* < 0.05).

**Supplemental Table 6. Food intake, growth, and organ weights** **in WKAH/HkmSlc male rats fed either control or CA diet treated with or without vancomycin (VCM;** **200 mg/L) in drinking water for 6 weeks (Study 3)**

 Two-way ANOVA *P*-value

 Control CA VCM CA + VCM CA VCM CA× VCM

Total food 661 ± 15 649 ± 19 619 ± 17 649 ± 20 0.61 0.25 0.26

intake g/6 weeks

Final body weight, g 279 ± 6.8 279 ± 7.9 257 ± 5.2 272 ± 9.3 0.34 0.053 0.33

Liver weight, g, 10.7 ± 0.4 11.7 ± 0.5 9.8 ± 0.9 11.0 ± 0.8 0.041 0.13 0.79

Epididymal adipose 6.5 ± 0.4 6.0 ± 0.2 5.3 ± 0.2 5.1 ± 0.3 0.26 0.001 0.61

tissue weight, g

Values are shown as the mean ± SEM (n = 8). Two-way ANOVA (CA and VCM) was used to evaluate differences.

**Supplemental Table 7.** **Aortic plasma BA profile** **in WKAH/HkmSlc male rats fed either control or CA diet treated with or without** **vancomycin (VCM; 200 mg/L) in drinking water for 6 weeks (Study 3)**

 Two-way ANOVA *P*-value

 Control CA VCM CA + VCM CA VCM CA× VCM

**Primary 12αOH BAs,** **nmol/L aortic plasma**

CA 2828 ± 541 2035 ± 804 287 ± 39 # 2673 ± 249\* 0.12 0.068 0.004

TCA 2951 ± 402 6994 ± 1524 2744 ± 154 9211 ± 1252 < 0.001 0.33 0.24

GCA 114 ± 20 148 ± 20 78 ± 10 1415 ± 316\*# < 0.001 < 0.001 < 0.001

**Secondary 12αOH BAs****, nmol/L aortic plasma**

DCA 232 ± 34 781 ± 186＊ 1.3 ± 1.3# 2.2 ± 2.2 # 0.008 < 0.001 0.008

TDCA 209 ± 186 971 ± 274＊ 0# 0 # 0.006 < 0.001 0.006

GDCA 8.2 ± 4.1 18 ± 4.7 0 0 0.11 < 0.001 0.11

7oDCA 52 ± 10 130 ± 49 16 ± 4.6 105 ± 19 0.003 0.27 0.72

12oLCA 0 7.4 ± 7.4 0 0 0.33 0.33 0.33

3o12α 12 ± 0.7 30 ± 6.7 0 0 0.074 < 0.001 0.074

UCA 59 ± 12 90 ± 29 2.8 ± 2.8 22 ± 52 0.13 < 0.001 0.78

**Primary non-12αOH BAs, nmol/L aortic plasma**

CDCA 107 ± 26 18 ± 12＊ 2.5 ± 2.5 # 3.9 ± 3.9 0.005 < 0.001 0.004

TCDCA 57 ± 6.2 25 ± 3.0 61 ± 7.2 36 ± 5.3 < 0.001 0.19 0.54

GCDCA 4.1 ± 4.1 0 0.3 ± 0.3 0 0.29 0.37 0.37

αMCA 163 ± 31 13 ± 8.9＊ 3.3 ± 3.3# 11 ± 7.0 < 0.001 < 0.001 < 0.001

βMCA 876 ± 104 883 ± 262 211 ± 18 398 ± 63 0.4 < 0.001 0.051

TαMCA 143 ± 17 68 ± 10 135 ± 15 87 ± 15 < 0.001 0.71 0.37

TβMCA 514 ± 62 383 ± 69 289 ± 29 275 ± 59 0.22 0.007 0.31

**Secondary non-12αOH BAs, nmol/L aortic plasma**

ωMCA 1566 ± 207 462 ± 67＊ 0# 0 # < 0.001 < 0.001 < 0.001

TωMCA 137 ± 14 19 ± 10＊ 0 # 0 < 0.001 < 0.001 < 0.001

HCA 0 0 0 0

HDCA 23 ± 11 0 0 0 0.048 0.048 0.048

THDCA 13 ± 3.9 0＊ 0 # 0 0.002 0.002 0.002

GHDCA 3.7 ± 3.7 0 0 0 0.33 0.33 0.33

UDCA 46 ± 9.0 15 ± 8.8 0 # 0 0.020 < 0.001 0.020

TUDCA 2.5 ± 2.5 0 0.4 ± 0.4 0 0.26 0.41 0.41

GUDCA 3.4 ± 3.4 0 0 0 0.33 0.33 0.33

LCA 15 ± 8.5 0 0 0 0.088 0.088 0.088

TLCA 13 ± 11 0 0 0 0.25 0.25 0.25

GLCA 6.1 ± 6.1 0 0 0 0.33 0.33 0.33

7oLCA 0 0 0 0

Values are shown as the mean ± SEM (n = 8). Values of undetectable parameters were considered to be 0 in the statistical analysis. Two-way ANOVA (CA and VCM) was used to evaluate differences. When there was a significant interaction (*P* < 0.05), further analysis of the simple effect by CA or vancomycin was then performed. The data were log-transformed (log10) to improve the homogeneity of the variance before ANOVA if needed. If there was an undetectable value in a parameter, all the values in the parameter were added 1 before the log-transformation. The means reported here were back-transformed for interpretation. \*Statistically significant effects of CA diet within the same treatment (*P* < 0.0125). #Statistically significant effects of vancomycin treatment within the same diet (*P* < 0.0125).

**Supplemental Table 8. Hepatic BA profile in** **WKAH/HkmSlc male rats fed either control or CA diet treated with or without** **vancomycin (VCM; 200 mg/L) in drinking water for 6 weeks (Study 3)**

 Two-way ANOVA *P*-value

 Control CA VCM CA + VCM CA VCM CA×VCM

**Primary 12αOH BAs, nmol/g liver**

CA 0.5 ± 0.1 0.3 ± 0.1 0.02 ± 0.01# 0.2 ± 0.04＊ 0.83 0.003 0.026

TCA 50 ± 4.4 95 ± 5.1＊ 48 ± 4.5 134 ± 9.5＊# < 0.001 0.007 0.003

GCA 0.7 ± 0.1 0.8 ± 0.05 0.6 ± 0.09 7.9 ± 2.3＊# < 0.001 < 0.001 < 0.001

**Secondary 12αOH BAs, nmol/g liver**

DCA 0 0.2 ± 0.1 0 0 0.22 0.22 0.22

TDCA 4.1 ± 0.4 16 ± 3.5＊ 0 # 0.01 ± 0.01# 0.028# < 0.001 0.031

GDCA 0.7 ± 0.2 0.2 ± 0.7 0 # 0.004 ± 0.004# 0.027 < 0.001 0.038

7oDCA 0.004 ± 0.004 0 0 0 0.33 0.33 0.33

12oLCA 0 0 0 0

3o12α 0 0 0 0

UCA 0.1 ± 0.06 0.02 ± 0.01 0 0 0.046 0.011 0.046

**Primary non-12αOH BAs, nmol/g liver**

CDCA 0 0 0 0

TCDCA 1.2 ± 0.1 0.7 ± 0.1 1.4 ± 0.2 0.9 ± 0.1 0.001 0.19 0.85

GCDCA 0.004 ± 0.004 0.001 ± 0.001 0.004 ± 0.004 0.002 ± 0.001 0.13 0.18 0.22

αMCA 0.3 ± 0.9 0＊ 0 # 0 0.007 0.007 0.007

βMCA 0.6 ± 0.2 0.2 ± 0.04 0.09 ± 0.04 0.04 ± 0.02 0.030 0.005 0.086

TαMCA 1.9 ± 0.3 1.5 ± 0.2 2.0 ± 0.3 1.3 ± 0.08 0.021 0.71 0.33

TβMCA 14 ± 2.1 11 ± 1.8 11 ± 1.3 5.9 ± 0.7 0.14 0.032 0.47

**Secondary non-12αOH BAs, nmol/g liver**

ωMCA 0.8 ± 0.2 0.2 ± 0.04＊ 0 # 0 # 0.003 < 0.001 0.003

TωMCA 5.4 ± 0.08 0.9 ± 0.1＊ 0 # 0.06 ± 0.02＊# < 0.001 < 0.001 < 0.001

HCA 0 0 0 0

HDCA 0 0 0 0

THDCA 0.2 ± 0.05 0.02 ± 0.01＊ 0.01 ± 0.01# 0.2 ± 0.1＊# 0.31 0.65 < 0.001

GHDCA 0 0.004 ± 0.004 0 0.003 ± 0.003 0.17 0.88 0.88

UDCA 0 0 0 0

TUDCA 0.5 ± 0.04 0.2 ± 0.03 0.5 ± 0.05 0.2 ± 0.01 < 0.001 0.68 0.50

GUDCA 0 0.005 ± 0.005 0 0 0.33 0.33 0.33

LCA 0 0.002 ± 0.002 0 0.01 ± 0.01 0.25 0.43 0.43

TLCA 0.2 ± 0.7 0.02 ± 0.007 0.001 ± 0.01# 0.01 ± 0.01 0.047 0.027 0.040

GLCA 0 0.01 ± 0.01 0 0.01 ± 0.01 0.18 0.77 0.77

7oLCA 0 0 0 0

Values are shown as the mean ± SEM (n = 8). Values of undetectable parameters were considered to be 0 in the statistical analysis. Two-way ANOVA (CA and VCM) was used to evaluate differences. When there was a significant interaction (*P* < 0.05), further analysis of the simple effect by CA or vancomycin was then performed. The data were log-transformed (log10) to improve the homogeneity of the variance before ANOVA if needed. If there was an undetectable value in a parameter, all the values in the parameter were added 1 before the log-transformation. The means reported here were back-transformed for interpretation. \*Statistically significant effects of CA diet within the same treatment (*P* < 0.0125). #Statistically significant effects of vancomycin treatment within the same diet (*P* < 0.0125).