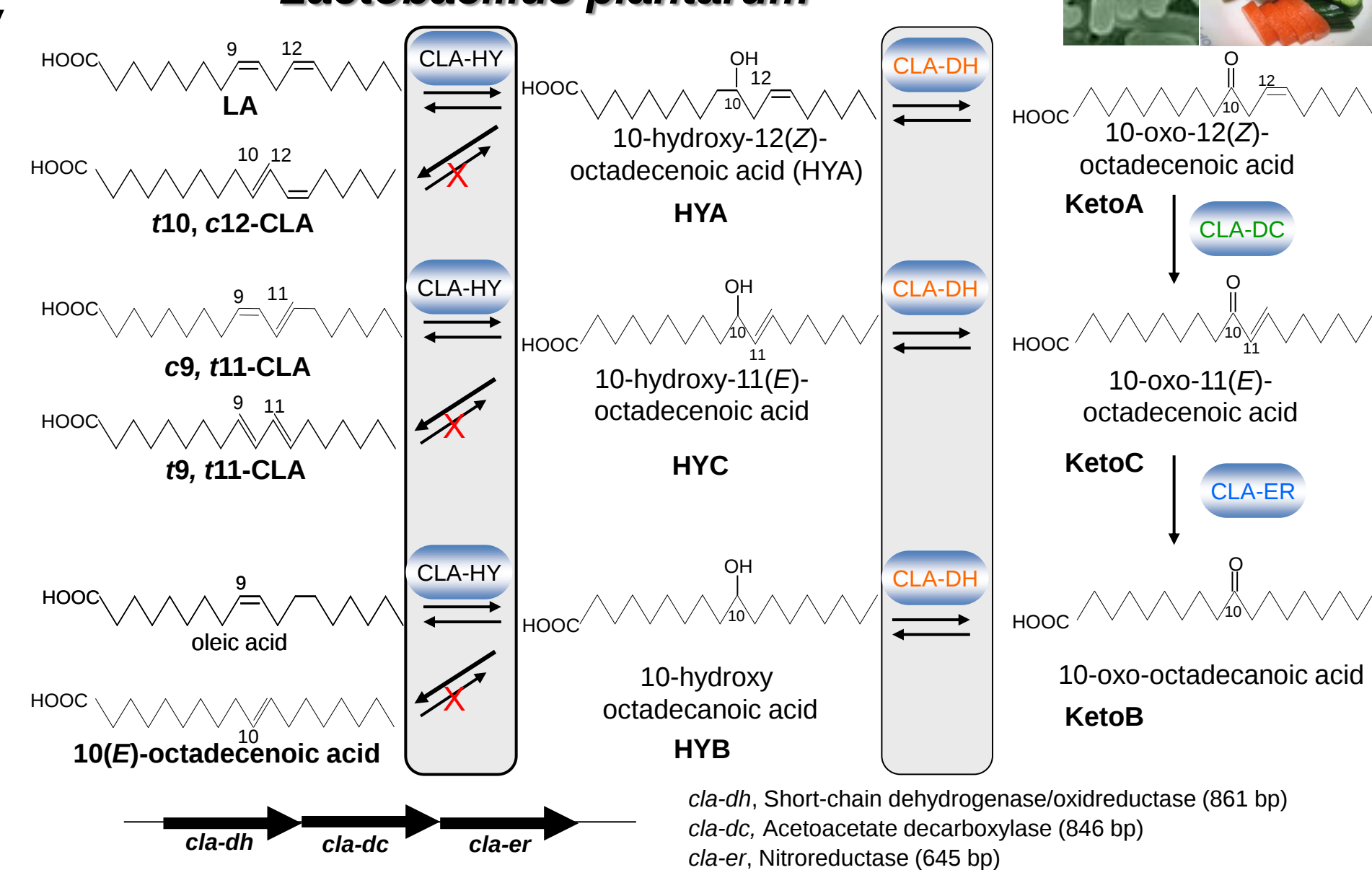


Division of Applied Life Sciences, Graduate School of Agriculture,

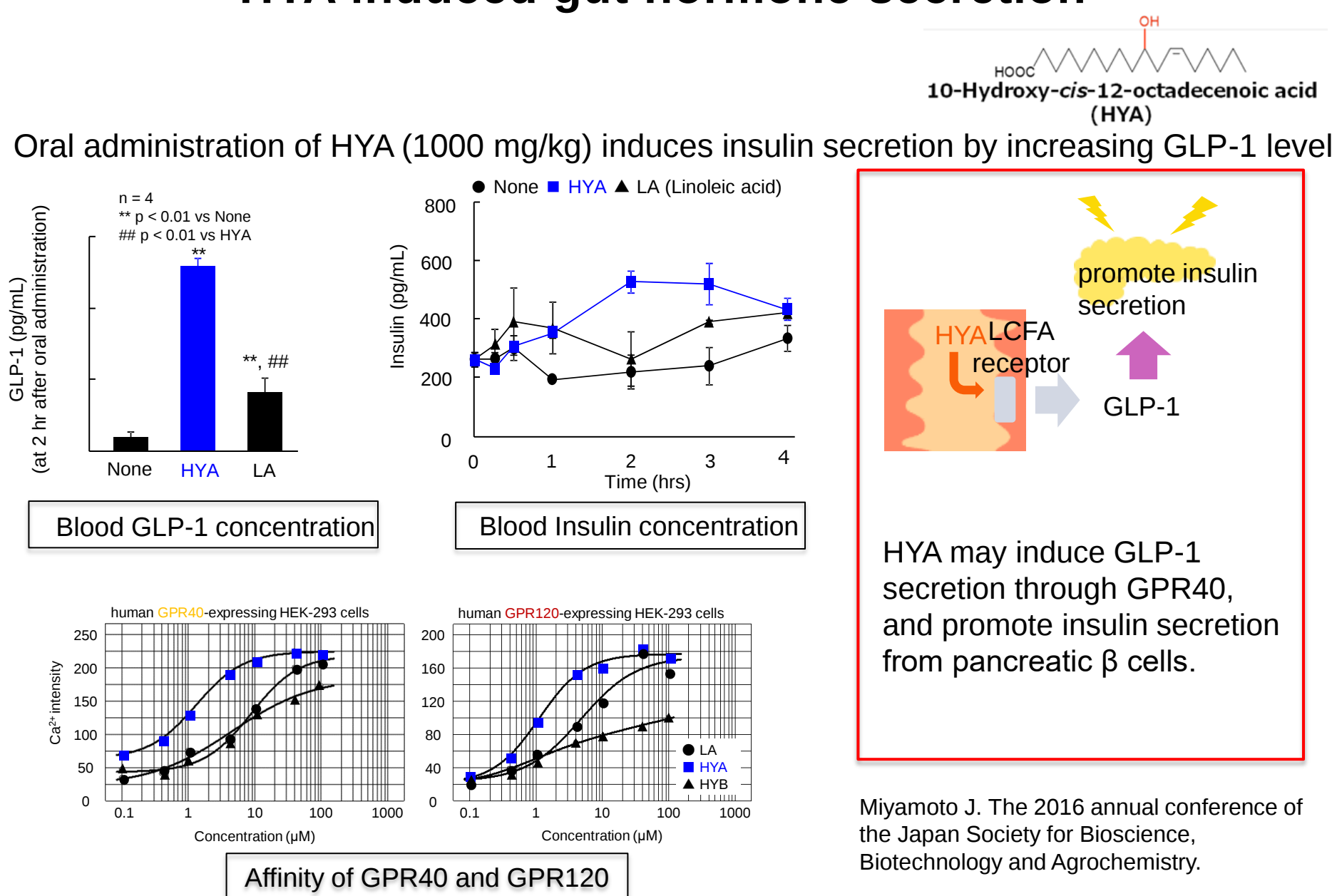
*E-mail: ogawa.jun.8a@kyoto-u.ac.jp, Tel: +81 75 753 6115, Fax: +81 75 753 6113

Prevalence of metabolic syndrome has stimulated interest in fat metabolism not only by the host but also by the gut microbiota. We revealed the dietary fatty acid metabolism in representative gut bacteria, lactic acid bacteria, generating hydroxy, oxo, enone, and conjugated fatty acids as intermediates. We confirmed the existence of these fatty acid metabolites in host tissues depending on the existence of gut bacteria and evaluated their physiological activity. For example, a linoleic acid-derived metabolite 10-hydroxy-cis-12-octadecenoic acid (HYA) induced gut hormone, GLP-1, secretion resulted in improving insulin resistance. HYA administration also altered gut microbiome profile bringing increased production of healthy gut microbial fatty acid metabolites. Thus, HYA acted as dual-controller repairing both disease conditions and gut microbial profiles. The effects of HYA was confirmed with human clinical test, then commercialized after development of its efficient microbial production process. HYA was proved to ameliorate sulfate sodium-induced colitis in mice by recovering the damage of intestinal epithelial barrier, and periodontal pathogen-induced gingival epithelial barrier disruption, via GPR40 signaling. Not only HYA, various gut microbial fatty acid metabolites were found to have health-promoting activities. For example, α -Linolenic acid-derived metabolites from gut lactic acid bacteria accumulate M2 macrophage in adipose tissue and repress inflammation. Dietary KetoA intake improved obesity-associated metabolic disorders via up-regulation of adipose tissue function by TRIPVI activation. These results suggested that the dietary fatty acid metabolites by gut microbiota can influence the health of the host. Gut microbial fatty acid metabolites might have potentials as novel functional foods, postbiotics, and pharmaceuticals.



Kishino, S. et al. *Proc. Natl. Acad. Sci. USA*, 110, 17808-17813 (2013)

Oral administration of HYA (1000 mg/kg) induces insulin secretion by increasing GLP-1 level



Miyamoto J., et al. NATURE COMMUNICATIONS 10:4007, 2019

Experimental Design Timeline:

- 4week old C57BL/6J σ^7
- 0 W
- 9-10 W
- 12 W
- harvest
- Blood glucose, Insulin
- analysis of gut microbes , metabolites in ileum

Control: HFD
HYA: 1%HYA+HFD
LA: 1%LA+HFD

Blood glucose (mg/dL)

Group	Blood glucose (mg/dL)
Control	~215
HYA	~195
LA	~205

Insulin (ng/mL)

Group	Insulin (ng/mL)
Control	~2.5
HYA	~1.2
LA	~1.5

ITT (Time to reach 180 mg/dL)

Group	Time (min)
Control	~15
HYA	~10
LA	~12

HYA improves HFD-induced insulin resistance by promoting GPR40 and 120-mediated GLP-1 secretion.

Miyamoto J., et al. NATURE COMMUNICATIONS 10:4007. 2019

[illegible]

Miyamoto J., et al. NATURE COMMUNICATIONS 10:4007. 2019

HYA increase the abundance of the *Lactobacillaceae* family and specific metabolites in ileum

The diagram illustrates the production of Hyaluronic Acid (HYA) from Linoleic acid (LA) via Lactic Acid Bacteria (LAB). It shows the following components and processes:

- Oil:** Represented by a beaker containing yellow oil.
- Lipase:** Indicated by a blue arrow pointing from the oil to the LAB, representing the enzymatic breakdown of oil into fatty acids.
- Lactic acid bacteria (LAB):** Shown as white, rod-shaped capsules.
- HYA:** Shown as a glass bottle containing a yellow liquid, representing the final product.
- Chemical Structures:**
 - Linoleic acid (LA):** A long-chain fatty acid with two double bonds (9 and 12).
 - HYA:** A complex molecule with multiple hydroxyl groups (OH) and carboxylic acid groups (HOOC).

The process involves the conversion of oil into fatty acids (LA) using lipase, followed by the action of Lactic Acid Bacteria (LAB) to produce Hyaluronic Acid (HYA).

Figure 1 DSS-induced colitis in mice and the effect of HYA on colitis. **A**, Timeline of DSS-induced colitis. Mice were treated with DSS (5.5%) for 10 days. **B**, Representative images of colon sections from untreated, DSS, DSS + HYA, and DSS + HYB mice. **C**, Histological score and colon length. **D**, TNFR2 signaling pathway. **E**, Relative mRNA expression of *tnfr2* in colon tissue.

A, Timeline of DSS-induced colitis. Mice were treated with DSS (5.5%) for 10 days. **B**, Representative images of colon sections from untreated, DSS, DSS + HYA, and DSS + HYB mice. **C**, Histological score and colon length. **D**, TNFR2 signaling pathway. **E**, Relative mRNA expression of *tnfr2* in colon tissue.

Table 1: Histological score and colon length

Group	Histological score	Colon length (cm)
Untreated	9.18 ± 0.2	~10.5
DSS	5.98 ± 0.14 ^{***}	~7.5
DSS + HYA	7.07 ± 0.12 ^{***,##}	~9.5
DSS + HYB	6 ± 0.18 ^{***,##,SS}	~9.0

Table 2: Relative mRNA expression of *tnfr2*

Group	Relative mRNA expression
Untreated	1.0
DSS (-)	~2.8 ^{**}
DSS + HYA	~1.8 [*]
DSS + HYB	~2.3 ^{**}

Table 3: Statistical significance

Comparison	Significance
Untreated vs DSS	***
DSS vs DSS + HYA	##
DSS vs DSS + HYB	SS
DSS + HYA vs DSS + HYB	ns

Figure 1D: TNFR2 signaling pathway

DSS-induced colitis is mediated by TNFR2 signaling. DSS treatment leads to TNF-α production, which binds to TNFR2, leading to down-regulation of TNFR2 and NF-κB suppression, resulting in recovery of intestinal barrier and anti-inflammation.

Figure 1E: Relative mRNA expression of *tnfr2*

Relative mRNA expression of *tnfr2* in colon tissue. The expression is significantly increased in DSS-treated mice compared to untreated mice. Treatment with HYA or HYB significantly reduces the expression of *tnfr2* in DSS-treated mice.

Figure 1F: DSS-induced colitis in mice and the effect of HYA on colitis

DSS-induced colitis in mice and the effect of HYA on colitis. DSS treatment leads to colitis, which is characterized by weight loss, diarrhea, and colon shortening. Treatment with HYA significantly reduces the severity of colitis, restoring weight, diarrhea, and colon length.

The Journal of Biological Chemistry, 290, 2902-2918 (2015)

Figure 1: Effects of HA on alveolar bone loss and cytokine production in a mouse model of periodontitis.

Panel A: Representative images of alveolar bone loss. The top row shows 'Unligated' and 'Ligated' (with *P. gingivalis* 10⁶ CFU) conditions. The bottom row shows 'Sham' and 'HYA' (hyaluronic acid) treated conditions. A blue arrow in the 'Ligated' image points to the alveolar bone crest.

Panel B: Bar graph of alveolar bone loss (ABC-CEJ distance* in μm).

Condition	Sham (Blue)	HYA (Red)
Unligated	~135	~125
Ligated	~280*	~190

* indicates statistical significance (p < 0.05) compared to the unligated Sham group.

Panel C: Bar graph of relative expression of inflammatory cytokines (TNF- α , IL-1 β , IL-6) in the gingiva (Unligated Sham=1).

Cytokine	Condition	Sham (Blue)	HYA (Red)
TNF- α	Unligated	1.0	1.0
	Ligated	~2.4*	~1.0
IL-1 β	Unligated	1.0	1.0
	Ligated	~7.5*	~3.2
IL-6	Unligated	1.0	1.0
	Ligated	~8.5*	~2.2

* indicates statistical significance (p < 0.05) compared to the unligated Sham group.

Panel D: Chemical structure of 10-Hydroxy-cis-12-octadecenoic acid (HYA).

Panel E: Summary of findings in a mouse periodontitis model. In a mouse periodontitis model, HYA suppressed the alveolar bone loss and subsequent inflammatory cytokine production in the gingival tissue.

In a mouse periodontitis model, HYA suppressed the alveolar bone loss and subsequent inflammatory cytokine production in the gingival tissue.

Scientific reports DOI:1038/s414598-018-27408-y (201

Methods

TRPV1-HEK293cells
(HEK293cells
overexpressing rTRPV1)
@40,000cells/ml

24h
@37°C

Fluo 4-AM (1µM)

1h
@37°C

LA derived FAs

30s~1min

measurement of fluorescence
by CLSM

Results

KetoA

CCCCCCCCCCCCCCCC(=O)C/C=C\CCCCCCCC

Compound	Fluorescence intensity (% of ionomycin)
LA	~5
CLA1	~20
CLA2	~8
CLA3	~18
10t	~22
HYA	~42
HYB	~18
HYC	~3
KetoA	~75
KetoB	n.d.
KetoC	~18
Cap	~100
ION	~100

fluorescence intensity
(% of ionomycin)

Conc (100µM)

n.d. : not detected
Cap : capsaicin
ION : ionomycin

The values are means ±SEM.

The values are means $\pm$ SEM.

Gene expression in iBAT and iWAT of WT or TRPV1 KO mice treated with KetoA

The diagram illustrates the experimental setup and results. A mouse is shown with iBAT (interscapular brown adipose tissue) and iWAT (inguinal white adipose tissue) locations marked. Arrows indicate the collection of these tissues. Below, bar graphs show the relative expression (%) of various genes in iBAT and iWAT for WT and TRPV1 KO mice, comparing control (white bars) and KetoA treatment (red bars). The y-axis represents 'relative expression (% of control)' from 0 to 200. The x-axis lists genes: rpl1, 3ar, c1a, t1b, tlea, and t16. In iBAT, KetoA treatment significantly increases expression of rpl1, 3ar, c1a, t1b, and tlea in WT mice (marked with *), while no significant change (NS) is observed in TRPV1 KO mice. In iWAT, KetoA treatment significantly increases expression of rpl1, 3ar, c1a, t1b, and tlea in WT mice (marked with *), while no significant change (NS) is observed in TRPV1 KO mice. A red circle highlights the significant increases in iBAT and iWAT for WT mice.

Legend: □ control ■ KetoA

Locations: iBAT, iWAT

Source: Bartelt A et al. *Nat Rev Endocrinol*. (2010)

iBAT

Gene	WT (control)	WT (KetoA)	TRPV1 KO (control)	TRPV1 KO (KetoA)
rpl1	100	~150*	~100	~100
3ar	100	~140*	~100	~100
c1a	100	~150*	~100	~100
t1b	100	~140*	~100	~100
tlea	100	~140*	~100	~100
t16	100	~200*	~100	~100

iWAT

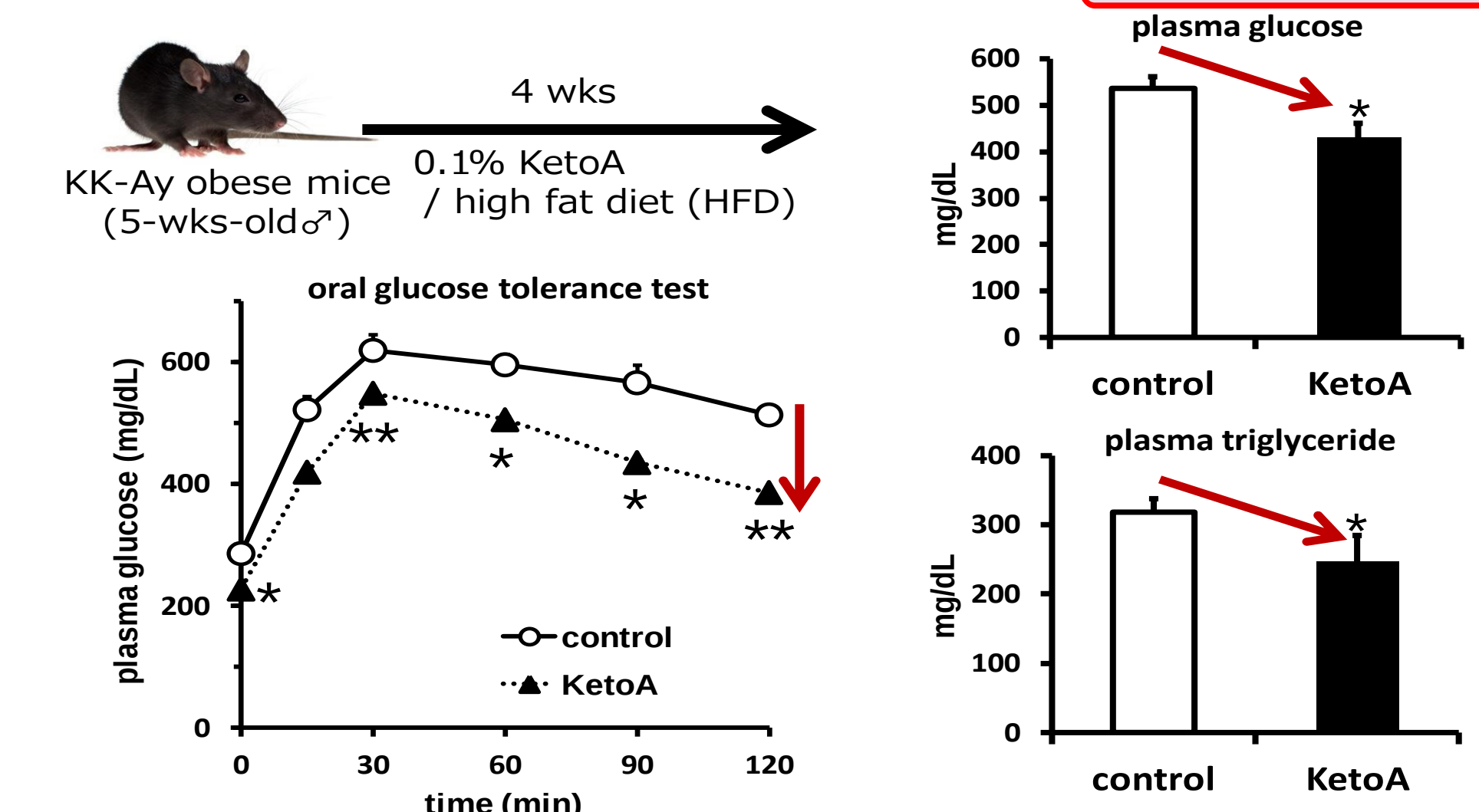
Gene	WT (control)	WT (KetoA)	TRPV1 KO (control)	TRPV1 KO (KetoA)
rpl1	100	~450*	~100	~100
3ar	100	~150*	~100	~100
c1a	100	~300*	~100	~100
t1b	100	~150*	~100	~100
tlea	100	~100	~100	~100
t16	100	~500*	~100	~100

KetoA treatment up-regulated BAT function in iBAT and iWAT and this function was dependent on TRPV1 activation.

Kim, M. et al. *EASEB J.* 31(11):5036-5048 (2017)

KetoA

CCCCCCCCCCCC(=O)C=CCCCCCCC



Dietary KetoA intake improved obesity-associated metabolic disorders via up-regulation of adipose tissue function by TRIPVI activation.

Kim, M. et al, *FASEB J.*, 31(11),5036-5048 (2017)