

## Supplementary Data for

### **Effects of Medium- and Long-chain Triacylglycerols on Lipid Metabolism and Gut Microbiota Composition in C57BL/6J Mice**

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## Tables

**Table S1.** Fatty acid compositions of RSO and MLCT (g/100g).

| FAC        | RSO           | MLCT-L       | MLCT-M       | MLCT-H       |
|------------|---------------|--------------|--------------|--------------|
| C8:0       | ND            | 5.5 ± 0.11b  | 10.7 ± 0.16c | 16.2 ± 0.17d |
| C10:0      | ND            | 4.5 ± 0.18b  | 9.3 ± 0.21c  | 13.8 ± 0.25d |
| C12:0      | ND            | ND           | ND           | ND           |
| C14:0      | ND            | ND           | ND           | ND           |
| C16:0      | 3.8 ± 0.20a   | 3.3 ± 0.12a  | 3.0 ± 0.15a  | 2.6 ± 0.09a  |
| C16:1      | 0.2 ± 0.01a   | 0.2 ± 0.01a  | 0.2 ± 0.01a  | 0.2 ± 0.01a  |
| C18:0      | 2.4 ± 0.05a   | 1.8 ± 0.07b  | 1.2 ± 0.05c  | 0.6 ± 0.04d  |
| C18:1      | 63.1 ± 0.52a  | 57.5 ± 0.78b | 51.2 ± 0.65c | 45.5 ± 0.46d |
| C18:2      | 18.8 ± 0.16a  | 16.9 ± 0.35b | 14.7 ± 0.38c | 12.4 ± 0.25d |
| C18:3      | 7.1 ± 0.07a   | 6.8 ± 0.11b  | 6.7 ± 0.17b  | 6.5 ± 0.13b  |
| C20:0      | 0.6 ± 0.01a   | 0.5 ± 0.02a  | 0.5 ± 0.02a  | 0.4 ± 0.02a  |
| C20:1      | 3.0 ± 0.03a   | 2.2 ± 0.05b  | 1.7 ± 0.06c  | 1.0 ± 0.01d  |
| C22:0      | 0.3 ± 0.01a   | 0.2 ± 0.01a  | 0.2 ± 0.02a  | 0.2 ± 0.01a  |
| C22:1      | 0.4 ± 0.02a   | 0.3 ± 0.02a  | 0.3 ± 0.03a  | 0.3 ± 0.02a  |
| C24:0      | 0.1 ± 0.01a   | 0.1 ± 0.01a  | 0.1 ± 0.01a  | 0.1 ± 0.01a  |
| C24:1      | 0.2 ± 0.01a   | 0.2 ± 0.02a  | 0.2 ± 0.02a  | 0.2 ± 0.01a  |
| Total MCFA | ND            | 10.0 ± 0.13a | 20.0 ± 0.17b | 30.0 ± 0.21c |
| Total LCFA | 100.0 ± 0.37a | 90.0 ± 0.32b | 80.0 ± 0.26c | 70.0 ± 0.25d |

ND stands for not detected; FAC stands for fatty acid compositions. Data are

expressed as mean  $\pm$  SD (n = 8). Values with different letters in the same row indicate significant differences ( $P < 0.05$ ).

**Table S2.** Triacylglycerols compositions of RSO and MLCT (g/100g).

| Triacylglycerols | RSO          | MLCT-L      | MLCT-M      | MLCT-H      |
|------------------|--------------|-------------|-------------|-------------|
| L-L-L            | 100.0 ± 0.0a | 52.4 ± 2.5b | 36.2 ± 1.4c | 4.5 ± 0.3d  |
| L-L-M/L-M-L      | ND           | 38.4 ± 1.2b | 24.3 ± 1.1c | 35.0 ± 0.8d |
| M-L-M/L-M-M      | ND           | 8.7 ± 0.5b  | 36.8 ± 0.9c | 56.5 ± 1.5d |
| M-M-M            | ND           | 0.5 ± 0.1b  | 2.7 ± 0.5c  | 4.0 ± 0.2d  |

L stands for long-chain fatty acids; M stands for medium-chain fatty acids; ND stands for not detected. Data are expressed as mean ± SD (n = 8). Values with different letters in the same row indicate significant differences ( $P < 0.05$ ).

**Table S3.** Sequences of real-time PCR primers.

| Gene name     | Direction | Primer sequence (5'-3')  |
|---------------|-----------|--------------------------|
| LCAD          | Forward   | CGCCCGATGTTCTCATTCT      |
|               | Reverse   | GGCTTTCTCCCATTCTTCGT     |
| MCAD          | Forward   | CGCCCCAGACTACGATAAAA     |
|               | Reverse   | CAAGACCACCACAACCTCTCC    |
| ME            | Forward   | CGGCACAGAAAATGAGGAGTT    |
|               | Reverse   | CCTTCTTGCTGCTCTTCGGAT    |
| PPAR $\alpha$ | Forward   | TACCACTATGGAGTCCACGCATGT |
|               | Reverse   | TTGCAGCTTCGATCACACTTGTCG |
| FAS           | Forward   | TCTGATCAGTGGCCTCCTTAAC   |
|               | Reverse   | CAGTGCTGAGATGTGGGAATAC   |
| ACC1          | Forward   | TGTAGAAACCCGAACCGTGG     |
|               | Reverse   | CTGGAAACCAAACCTTGGCCG    |
| SREBP-1c      | Forward   | TGGAGCGAGCATTGAACTGT     |
|               | Reverse   | GTGGTAGCCATGCTGGAACT     |

LCAD stands for long-chain acyl-CoA dehydrogenase; MCAD stands for medium-chain acyl-CoA dehydrogenase; ME stands for malic enzyme; PPAR $\alpha$  stands for peroxisome proliferator activated receptor-alpha; FAS stands for fatty acid synthase; ACC1 stands for acetyl-CoA carboxylase 1; SREBP-1c stands for sterol regulatory element binding protein-1c.

**Table S4.** Food composition (g) of each C57BL/6J mouse group (n = 8) during the 6-week period study.

| Week | LFD           | HFD           | MLCT-L         | MLCT-M        | MLCT-H         |
|------|---------------|---------------|----------------|---------------|----------------|
| 1    | 21.56 ± 2.87a | 18.34 ± 2.71a | 17.99 ± 2.56a  | 18.34 ± 2.34a | 19.46 ± 1.98a  |
| 2    | 18.76 ± 2.12a | 18.69 ± 2.31a | 16.59 ± 1.87ab | 14.28 ± 1.98b | 18.48 ± 2.03a  |
| 3    | 18.48 ± 2.21a | 16.66 ± 1.97a | 15.05 ± 2.01ab | 13.65 ± 1.89b | 15.75 ± 2.11ab |
| 4    | 18.76 ± 2.25a | 16.66 ± 1.89a | 14.70 ± 2.05ab | 14.00 ± 1.97b | 14.98 ± 2.03ab |
| 5    | 22.12 ± 2.63a | 19.60 ± 2.41a | 17.92 ± 2.65ab | 16.87 ± 2.26b | 17.85 ± 2.31ab |
| 6    | 21.77 ± 2.75a | 17.71 ± 2.42a | 17.78 ± 2.53a  | 16.80 ± 2.42a | 17.99 ± 2.36a  |

Data are expressed as mean ± SD (n = 8). Values with different letters in the same row indicate significant differences ( $P < 0.05$ ).

**Table S5.** Richness and diversity indexes from fecal samples of each group (OTU cutoff of 0.03).

|              | LFD            | HFD           | MLCT-L         | MLCT-M         | MLCT-H         |
|--------------|----------------|---------------|----------------|----------------|----------------|
| Reads        | 2947 ± 440a    | 3193 ± 475a   | 3049 ± 448a    | 3378 ± 392a    | 3553 ± 498a    |
| OTUs         | 787 ± 89ab     | 786 ± 149ab   | 740 ± 111b     | 947 ± 134a     | 844 ± 132ab    |
| Coverage (%) | 0.79 ± 0.02a   | 0.81 ± 0.06a  | 0.81 ± 0.04a   | 0.78 ± 0.03a   | 0.81 ± 0.05a   |
| Ace          | 6099 ± 860a    | 5890 ± 1732a  | 6381 ± 1059a   | 7107 ± 1901a   | 6327 ± 1788a   |
| Chao         | 2892 ± 376a    | 2828 ± 773a   | 2870 ± 452a    | 3545 ± 689a    | 3038 ± 692a    |
| Shannon      | 4.33 ± 0.13a   | 4.42 ± 0.61a  | 4.08 ± 0.35a   | 4.40 ± 0.33a   | 4.04 ± 0.38a   |
| Simpson      | 0.088 ± 0.019a | 0.057 ± 0.09a | 0.111 ± 0.039a | 0.116 ± 0.044a | 0.138 ± 0.035a |

Data are expressed as mean ± SD (n = 8). Values with different letters in the same row indicate significant differences ( $P < 0.05$ ).

**Table S6.** Fecal bacterial composition at the phylum level after 6-weeks feeding.

|                 | LFD           | HFD           | MLCT-L        | MLCT-M        | MLCT-H                    |
|-----------------|---------------|---------------|---------------|---------------|---------------------------|
| Actinobacteria  | 0.11 ± 0.05a  | 0.37 ± 0.06b  | 0.28 ± 0.04bc | 0.22 ± 0.03c  | 0.08 ± 0.03a              |
| Bacteroidetes   | 69.40 ± 5.40a | 36.70 ± 3.50b | 50.76 ± 3.52c | 72.26 ± 4.61a | 57.87 ± 2.46 <sup>d</sup> |
| Firmicutes      | 29.54 ± 3.13a | 59.30 ± 4.83b | 47.85 ± 3.61c | 23.44 ± 3.29d | 41.34 ± 3.87e             |
| Unspecified     | 0.33 ± 0.08a  | 0.63 ± 0.11b  | 0.42 ± 0.06c  | 0.94 ± 0.12d  | 0.34 ± 0.05a              |
| Proteobacteria  | 0.19 ± 0.06a  | 2.45 ± 0.66b  | 0.19 ± 0.07a  | 0.74 ± 0.11c  | 0.15 ± 0.04a              |
| Tenericutes     | 0.37 ± 0.07a  | 0.11 ± 0.04b  | 0.20 ± 0.06b  | 0.39 ± 0.08a  | 0.18 ± 0.04b              |
| Verrucomicrobia | 0.08 ± 0.03a  | 0.19 ± 0.05b  | 0.27 ± 0.07b  | 0.05 ± 0.02ac | 0.02 ± 0.01c              |

Data are expressed as mean ± SD (n = 8). Values with different letters in the same row

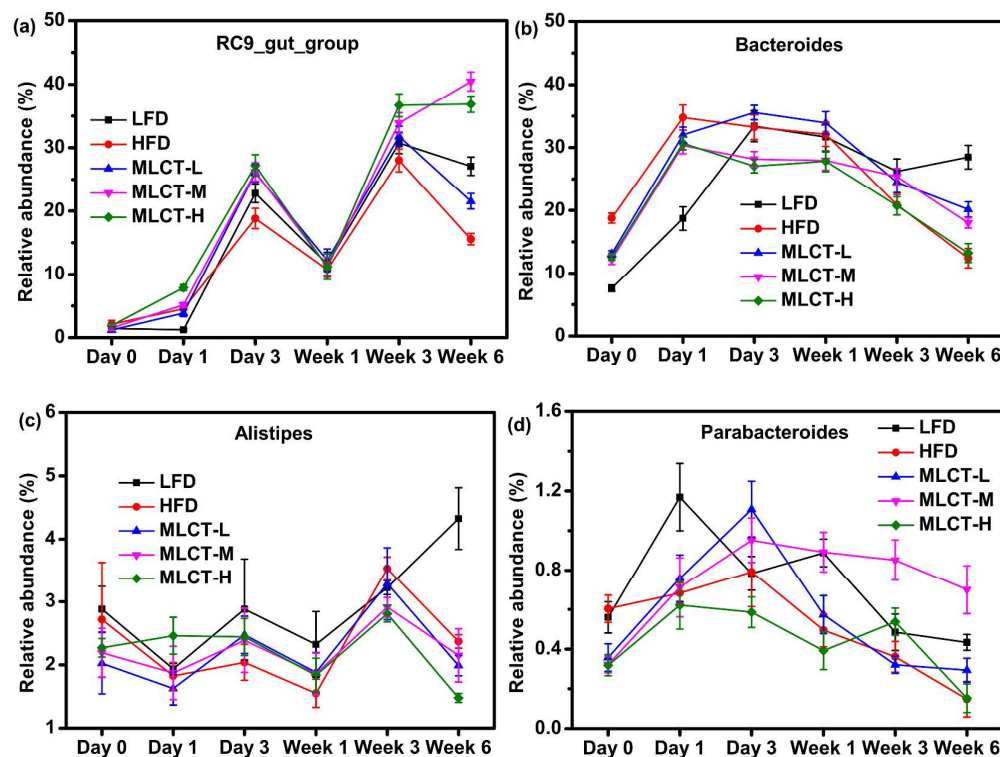
indicate significant differences ( $P < 0.05$ ).

**Table S7.** The ratio of *Firmicutes* to *Bacteroidetes* (F/B) after 6-weeks feeding.

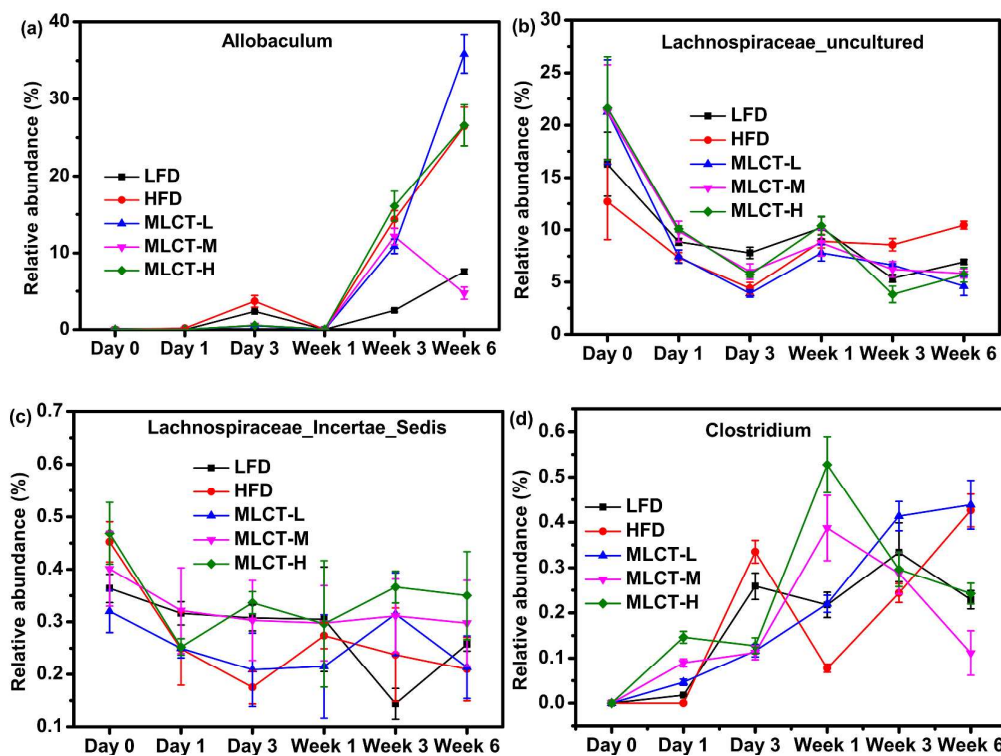
|     | LFD          | HFD          | MLCT-L       | MLCT-M       | MLCT-H       |
|-----|--------------|--------------|--------------|--------------|--------------|
| F/B | 0.43 ± 0.14a | 1.46 ± 0.25b | 0.94 ± 0.12c | 0.34 ± 0.08a | 0.56 ± 0.16a |

Data are expressed as mean ± SD (n = 8). Values with different letters in the same row indicate significant differences ( $P < 0.05$ ).

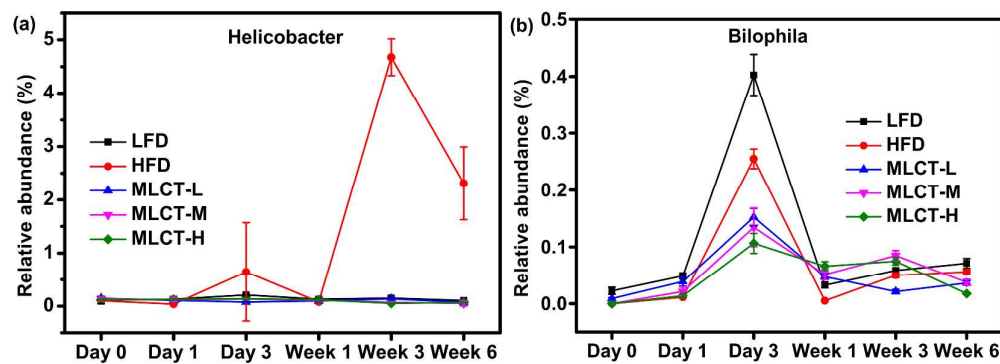
## Figures



**Figure S1.** Variation of key genera belonging to *Bacteroidetes* during 6-weeks study (n = 8).



**Figure S2.** Variation of key genera belonging to *Firmicutes* during 6-weeks study (n = 8).



**Figure S3.** Variation of key genera belonging to *Proteobacteria* during 6-weeks study (n = 8).