Supplementary Table 1. Composition of the experimental diets (calculated values).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | HFI | HFR | HFR | HFR |
|  | 100% ad libitum | 75% ad libitum | 60% ad libitum | 50% ad libitum |
| Ingredients (g/kg) |  |  |  |  |
| Casein HCl | 195 | 256 | 322 | 379 |
| L-Cystine | 2.50 | 3.28 | 4.13 | 4.86 |
| Corn starch | 250 | 196 | 135 | 87 |
| Sucrose | 142 | 112 | 78 | 50 |
| Oils \* | 295 | 295 | 300 | 302 |
| Alpha-cellulose | 50.3 | 50.21 | 50.1 | 50.0 |
| AIN 93M Mineral mix | 48.5 | 63.7 | 80.2 | 94.4 |
| AIN 93VX Vitamin mix | 13.9 | 18.2 | 23.0 | 27.0 |
| Choline chloride | 2.78 | 3.64 | 4.59 | 5.40 |
| Energy |  |  |  |  |
| Total (MJ/kg) | 20.17 | 19.89 | 19.70 | 19.62 |
| Protein (%) | 15.6 | 20.6 | 26.4 | 31.3 |
| Lipid (%) | 54.5 | 55.6 | 56.8 | 57.8 |
| Carbohydrate (%) | 29.9 | 23.8 | 16.8 | 10.8 |

HFI, high-fat induction diet; HFR, high-fat restriction diet.

\*: blend of 78.4% high-oleic sunflower oil and 21.6% sunflower oil in the HFI- and HFR-OLE diets, rich in oleic acid (18:1 n-9); 100% rapeseed oil in the HFI- and HFR-ALA diets, rich in a-linolenic acid (18:3 n-3); 33.2% fish oil, 40.3% high-oleic sunflower oil and 26.5% sunflower oil in the HFI- and HFR-LC diets, rich in n-3 long-chain polyunsaturated fatty acids.

Supplementary Table 2. Primer sequences used in quantitative RT-PCR analysis.

|  |  |  |
| --- | --- | --- |
| Gene name | Forward primer | Reverse primer |
| HPRT | TCCATTCCTATGACTGTAGATTTTATCAG | AAATTTTATGTCCCCCGTTGACT |
| Fbxo32 | GGACTTCTCGACTGCCATCCT | CAGCTCCAACAGCCTTACTACGT |
| Murf1 | GGTGCCTACTTGCTCCTTGTG | CAGTTCAGTCTTCTGTCCTTGGAA |
| Psma1 | AGAAAGACCACAGAGAAAAGC | TACCACCCAATACATTACAGC |
| Ubb | CGCACCCTCTCTGACTACA | GCCCTCTTTATCCTGGATCT |
| Capn2 | GCTGGAGGAAGAAGATGAAG | GTTTTTGCTGAGGTGGATG |
| Ctsd | ACACTGGCTCCTCTAACCTG | TCCACCTTGATACCTCCTAAG |
| InsR | CCAATGGCAACATCACACACTA  | TTCAGCCCTTTGAGACAATAATCC  |

HPRT, hypoxanthine phosphoribosyltransferase; Fbxo32, F-box protein 32 (or MAFbx); MuRF1, muscle ring finger-1 (or E3 ubiquitin protein ligase); Psma1, proteasome subunit, alpha type 1; Ubb, ubiquitin B; Capn2, calpain-2; Ctsd, cathepsin D; InsR, insulin receptor.

Supplementary Table 3. Fatty acid composition (mol/100 mol) of polar lipids in the *Gastrocnemius* muscle after a 8-week energy restriction.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | OLE-AL | OLE-R | ALA-R | LC-R | Restriction | Dietary FA |
| C16:0 | 17.21±0.40bc | 17.11±0.27bc | 17.85±0.35ac | 18.69±0.47a\* | 0.1318 | 0.0180 |
| C18:0 | 14.99±0.28a | 15.02±0.18a | 13.12±0.19b\*\*\* | 13.61±0.20b\*\*\* | 0.0001 | <0.0001 |
| C22:0 | 1.70±0.20 | 1.69±0.26 | 1.77±0.24 | 2.01±0.18 | 0.6376 | 0.5865 |
| Total SFA | 34.28±0.34ac | 34.20±0.18ac | 33.18±0.27bc | 34.88±0.51a | 0.6308 | 0.0038 |
| C16:1 n-9 | 0.36±0.01a | 0.30±0.01b | 0.31±0.01b\* | 0.27±0.01b\*\*\* | 0.0003 | 0.2356 |
| C16:1 n-7 | 0.26±0.02b | 0.23±0.02b | 0.28±0.02b | 0.53±0.04a\*\*\* | 0.0172 | <0.0001 |
| C18:1 n-9c | 13.09±0.26a | 13.15±0.60a | 11.65±0.36ac | 10.90±0.54bc\*\* | 0.0344 | 0.0044 |
| C18:1 n-7 | 2.53±0.0b | 2.55±0.06b | 3.63±0.07a\*\*\* | 2.63±0.07b | <0.0001 | <0.0001 |
| C20:1 n-9 | 0.17±0.01b | 0.17±0.04b | 0.26±0.01a\* | 0.14±0.01b | 0.4960 | 0.0008 |
| Total MUFA | 16.45±0.30 | 16.48±0.68 | 16.22±0.43 | 14.53±0.61\* | 0.2642 | 0.0282 |
| C18:2 n-6c | 13.94±1.25c | 15.46±1.36b\*\* | 17.08±0.80a\*\*\* | 18.15±0.79a\*\*\* | <0.0001 | <0.0001 |
| C20:3 n-6 | 0.46±0.05 | 0.46±0.04 | 0.52±0.05 | 0.50±0.16 | 0.2725 | 0.2233 |
| C20:4 n-6 | 17.86±0.38a | 17.52±0.39a | 13.31±0.23b\*\*\* | 7.40±0.242c\*\*\* | <0.0001 | <0.0001 |
| C22:4 n-6 | 1.03±0.03a | 0.83±0.02b\*\*\* | 0.29±0.01c\*\*\* | 0.14±0.03d\*\*\* | <0.0001 | <0.0001 |
| C22:5 n-6 | 2.72±0.20a | 1.99±0.16b\*\*\* | 0.30±0.05c\*\*\* | 0.34±0.03c\*\*\* | <0.0001 | <0.0001 |
| Total n-6 | 36.36±0.41a | 36.53±0.56a | 31.75±0.32b\*\*\* | 27.61±0.29c\*\*\* | <0.0001 | <0.0001 |
| LC n-6 | 22.23±0.49a | 20.88±0.40a\* | 14.53±0.19b\*\*\* | 9.18±0.30c\*\*\* | <0.0001 | <0.0001 |
| C18:3 n-3 | 0.05±0.0b | 0.05±0.01b | 0.48±0.03a\*\*\* | 0.10±0.02b | <0.0001 | 0.0001 |
| C20:3 n-3 | 1.02±0.11 | 0.99±0.08 | 1.07±0.12 | 1.07±0.10 | 0.8386 | 0.8339 |
| C20:5 n-3 | 0.20±0.03b | 0.16±0.04b | 0.31±0.02b | 1.04±0.07a\*\*\* | <0.0001 | <0.0001 |
| C22:5 n-3 | 1.08±0.05c | 1.07±0.04c | 2.77±0.11a\*\*\* | 1.78±0.04b\*\*\* | <0.0001 | <0.0001 |
| C22:6 n-3 | 10.52±10.43c | 10.41±0.26c | 14.19±0.20b\*\*\* | 18.93±0.66a\*\*\* | <0.0001 | <0.0001 |
| Total n-3 | 12.91±0.48c | 12.79±0.27c | 18.85±0.22b\*\*\* | 23.08±0.71a\*\*\* | <0.0001 | <0.0001 |
| LC n-3 | 12.88±0.48c | 12.75±0.27c | 18.37±0.23b\*\*\* | 22.98±0.72a\*\*\* | <0.0001 | <0.0001 |
| Total PUFA | 49.27±0.48 | 49.32±0.65 | 50.60±0.29 | 50.59±0.74 | 0.1897 | 0.1837 |

Values are means ± SD for 11-12 rats per group. Only fatty acids (FA) accounting for more than 1% or with physiologically relevant are given. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; LC, long chain; ND, not detected (detection limit 0.1-0.2% of total FA). AL, *ad libitum*; R, restricted; OLE, diet rich in oleic acid (18:1 n-9); ALA, diet rich in -linolenic acid (18:3 n-3); LC, diet rich in n-3 long-chain polyunsaturated fatty acids.

Mean values within a row sharing a same superscript letter or without superscript letter were not significantly different at *P*<0.05 according to *post-hoc* Tukey-Kramer analysis.

\*, p<0.05; \*\*, p<0.01; \*\*\*, p<0.001 compared to the OLE-AL group according to Dunnett’s test.

Supplementary Table 4. Fatty acid composition (mol/100 mol) of neutral lipids in the *Gastrocnemius* muscle after a 8-week energy restriction.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | OLE-AL | OLE-R | ALA-R | LC-R | Restriction | Dietary FA |
| C14:0 | 0.66±0.02b | 0.58±0.02b | 0.63±0.02b | 1.33±0.03a\*\*\* | <0.0001 | <0.0001 |
| C16:0 | 13.44±0.24b | 12.84±0.26b | 13.69±0.32b | 15.84±0.26a\*\*\* | 0.1099 | 0.0006 |
| C18:0 | 4.59±0.22 | 4.86±0.23 | 4.18±0.15 | 4.67±0.13 | 0.5309 | 0.3721 |
| Total SFA | 19.55±0.48b | 19.10±0.44b | 19.61±0.45b | 23.02±0.36a\*\*\* | 0.0864 | 0.0065 |
| C16:1 n-7 | 1.66±0.12b | 1.42±0.08b | 1.73±0.15b | 2.88±0.13a\*\*\* | 0.3106 | 0.0004 |
| C18:1 n-9c | 51.53±0.68a | 51.30±0.92a | 44.62±0.52b\*\*\* | 40.95±0.37b\*\*\* | 0.0002 | <0.0001 |
| C18:1 n-7 | 2.18±0.04b | 2.28±0.21b | 4.09±005a\*\*\* | 3.20±0.08a\* | 0.004 | <0.0001 |
| Total MUFA | 56.37±0.76a | 56.04±0.80a | 52.03±0.535b\*\*\* | 48.10±0.40b\*\*\* | 0.0013 | 0.0003 |
| C18:2 n-6c | 18.58±0.31b | 18.84±0.29b | 19.61±0.17b | 21.61±0.35a\*\*\* | 0.0217 | <0.0001 |
| C20:3 n-6 | 0.13±0.01 | 0.14±0.01 | 0.16±0.01 | 0.17±0.01 | 0.1293 | 0.8622 |
| C20:4 n-6 | 2.51±0.24ab | 2.76±0.27a | 2.33±0.11ab | 1.33±0.08b | 0.5374 | 0.0129 |
| C22:4 n-6 | 0.32±0.01a | 0.30±0.02a | 0.17±0.01b\*\* | 0.14±0.02b\*\*\* | 0.0021 | <0.0001 |
| C22:5 n-6 | 0.36±0.04a | 0.31±0.03a | 0.02±0.014b\*\*\* | 0.14±0.03b\*\*\* | 0.0003 | <0.0001 |
| Total n-6 | 22.19±0.31ab | 22.61±0.32b | 22.52±0.22ab | 23.76±0.32a\* | 0.2186 | 0.0250 |
| LC n-6 | 3.45±0.27ab | 3.64±0.31a | 2.81±0.13ab | 1.94±0.10b\* | 0.2736 | 0.0088 |
| C18:3 n-3 | 0.33±0.06b | 0.27±0.03b | 2.66±0.09a\*\*\* | 0.40±0.02b | <0.0001 | <0.0001 |
| C20:5 n-3 | 0.08±0.01b | 0.12±0.02b | 0.12±0.01b | 0.56±0.04a\*\*\* | <0.0001 | <0.0001 |
| C22:5 n-3 | 0.14±0.01b | 0.16±0.01b | 0.57±0.03a\*\*\* | 0.76±0.04a\*\*\* | <0.0001 | <0.0001 |
| C22:6 n-3 | 0.83±0.10b | 1.11±0.12bc | 1.75±0.11ac\*\*\* | 2.58±0.20a\*\*\* | 0.0005 | 0.0041 |
| Total n-3 | 1.89±0.19b | 2.25±0.17b | 5.84±0.22a\*\*\* | 5.12±0.28a\*\*\* | <0.0001 | <0.0001 |
| LC n-3 | 1.56±0.16b | 1.97±0.18bc | 3.15±0.26ac\*\*\* | 4.64±0.28a\*\*\* | 0.0003 | 0.0005 |
| Total PUFA | 24.08±0.41b | 24.86±0.43b | 28.36±0.30a\*\*\* | 28.87±0.32a\*\*\* | <0.0001 | <0.0001 |

Values are means ± SD for 11-12 rats per group. Only fatty acids (FA) accounting for more than 1% or with physiologically relevant are given. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; LC, long chain; ND, not detected (detection limit 0.1-0.2% of total FA) AL, *ad libitum*; R, restricted; OLE, diet rich in oleic acid (18:1 n-9); ALA, diet rich in -linolenic acid (18:3 n-3); LC, diet rich in n-3 long-chain polyunsaturated fatty acids.

Mean values within a row sharing a same superscript letter or without superscript letter were not significantly different at *P*<0.05 according to *post-hoc* Tukey-Kramer analysis.

\*, p<0.05; \*\*, p<0.01; \*\*\*, p<0.001 compared to the OLE-AL group according to Dunnett’s test.

Supplementary Figure 1. Relative phosphorylation level of *Gastrocnemius* muscle proteins involved in insulin signaling.

 

(-) rats receiving an injection of saline solution; (+) rats receiving an injection of insulin at 1UI/Kg; IRS1, insulin receptor substrate 1; AKT, protein kinase B (PKB); mTOR, mammalian target of rapamycin; S6RP, phosphorylated S6 ribosomal protein. AL, *ad libitum*; R, restricted; OLE, diet rich in oleic acid (18:1 n-9); ALA, diet rich in -linolenic acid (18:3 n-3); LC, diet rich in n-3 long-chain polyunsaturated fatty acids.