Effects of moderate chronic food restriction on the development of postprandial dyslipidemia with ageing

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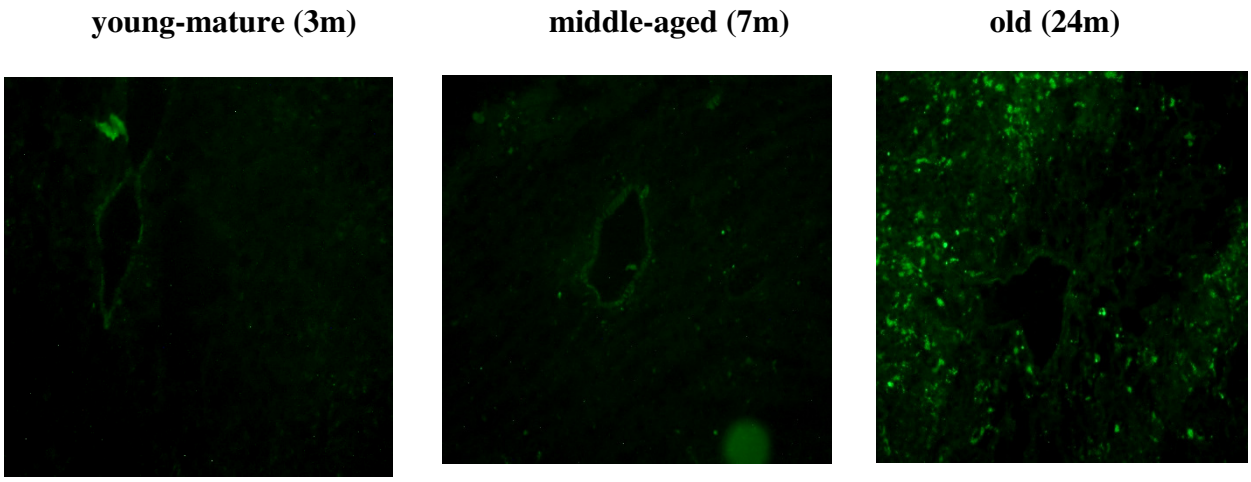
SUPPLEMENTARY MATERIALS

Lipofuscin determination

The presence of lipofuscin, a hallmark of senescent cells and particularly of liver oxidative stress, steatosis and fibrosis, was analyzed in unfixed, unstained cryostatic (30 μm thick) liver section from young (3m), mature middle-aged (7m) and middle-old age (24m) Wistar rats. Hepatic lipofuscin and lipofuscin-like pigments autofluorescence was measured at 510-580 nm, with excitation at 488 nm, and was visualized by fluorescent microscopy (Zeiss ICS Standart 25).



Supplementary Figure S1. Lipofuscin accumulation in hepatic senescent cells with ageing. Four rats from each group were used and 3 images from each rat were analyzed. Representative microphotograps showing autofluorescence detection of the intracellular accumulation of lipofuscin and lipofuscin-like pigments in the liver with ageing.

Supplementary Table S1. Macronutrients of rodent maintenance diet 2014 Teklad Global 14% protein.

Macronutrients	units	quantity
raw protein	%	14.5
lipids	%	4.0
carbohydrates	%	55.5
raw fibers	%	4.5
ash	%	4.7
Metabolizable energy	Kcal/g	3.1
Calories from protein	%	18
Calories from lipids	%	11
Calories from carbohydrates	%	71

Supplementary information to data in Figure 1. Summary of the effects of ageing, diet (AL or FR) and their interactions, obtained by two-way ANOVA followed by Tukey´s post hoc test, on the postprandial levels of glucose, insulin and glucagon in response to OLLT. Statistical significance was set at $p \leq 0.05$.

Serum levels	AGE	DIET	AGE & DIET
Glucose AUC	F(1,20)=0.4546 **p= 0.5079, ns**	F(1,20)=11.84 p= 0.0026	F(1,20)=44.04 p≤0.0001
Insulin AUC	F(1,20)=49.15 p≤0.0001	F(1,20)=5.96 **p= 0.052, ns**	F(1,20)=16.92 p= 0.0024
Glucagon AUC	F(1,20)=27.58 p≤0.0001	F(1,20)=18.30 p= 0.0004	F(1,20)=8.11 p= 0.0099

Supplementary information to data in Figure 2. Summary of the effects of ageing, diet (AL or FR) and their interactions, obtained by two-way ANOVA followed by Tukey´s post hoc test, on the postprandial levels of TG, NEFA, glycerol and ketone bodies in response to OLLT. Statistical significance was set at $p \leq 0.05$.

Serum levels	AGE	DIET	AGE & DIET
TG AUC	F(1,20)=557.9 p≤0.0001	F(1,20)=1802 p≤0.0001	F(1,20)=404.6 p≤0.0001
NEFA AUC	F(1,20)=10.87 p= 0.0036	F(1,20)=299.5 p≤0.0001	F(1,20)=103.3 p≤0.0001
Glycerol AUC	F(1,20)=184.2 p≤0.0001	F(1,20)=337.4 p≤0.0001	F(1,20)=69.78 p≤0.0001
KB AUC	F(1,20)=18.11 p= 0.0004	F(1,20)=1106 p≤0.0001	F(1,20)=8.96 p= 0.0072

Supplementary information to data in Figure 3. Summary of the effects of ageing, diet AL or FR) and their interactions, obtained by two-way ANOVA followed by Tukey´s post hoc test, on the postprandial levels of TG, NEFA and glycerol in response to OLLT. Statistical significance was set at $p \leq 0.05$.

Serum levels	AGE	DIET	AGE & DIET
Total Cholesterol AUC	F(1,20)=4.44 p= 0.0479	F(1,20)=56.28 p≤0.0001	F(1,20)=16.50 p= 0.0006
HDL- Cholesterol AUC	F(1,20)=59.24 p≤0.0001	F(1,20)=0.78 **p= 0.3853, ns**	F(1,20)=0.34 **p= 0.5642, ns**

Supplementary information to data in Figure 4. Summary of the effects of ageing, diet (AL or FR), OLLT and their interactions, obtained by three-way ANOVA followed by Tukey´s post hoc test, on the expression of liver genes involved in lipid uptake and oxidation. Statistical significance was set at p≤0.05.

Gene	OLLT	AGE	DIET	OLLT & AGE	OLLT & DIET	AGE & DIET	OLLT, AGE & DIET
LRP-1	F(1,50)=19.36 p≤0.0001	F(1,50)=2.74 **p = 0.104, ns**	F(1,50)=216.9 p≤0.0001	F(1,50)=133.5 p≤0.0001	F(1,50)=1.147 **p = 0.289, ns**	F(1,50)=103.5 p≤0.0001	F(1,50)=2.134 **p = 0.150, ns**
PPARα	F(1,50)=553 p≤0.0001	F(1,50) =2039 p≤0.0001	F(1,50) =85.30 p≤0.0001	F(1,50)=1639 p≤0.0001	F(1,50) =72.24 p≤0.0001	F(1,50) =0.009 p = 0.987	F(1,50) =2658 p≤0.0001
PGC-1α	F(1,48)=74.95 p≤0.0001	F(1,48)=186.0 p≤0.0001	F(1,48)=81.07 p≤0.0001	F(1,48)=5.83 p= 0.0196	F(1,48)=17.02 p≤0.0001	F(1,48)=104.1 p≤0.0001	F(1,48)=5.34 p= 0.0249
CPT-1a	F(1,48)=3876 p≤0.0001	F(1,48)=1610 p≤0.0001	F(1,48)=1428 p≤0.0001	F(1,48)=1161 p≤0.0001	F(1,48)=1046 p≤0.0001	F(1,48)=1365 p≤0.0001	F(1,48)=1003 p≤0.0001
MCAD	F(1,50)=6.92 p= 0.0113	F(1,50)=128.9 p≤0.0001	F(1,50)=321.9 p≤0.0001	F(1,50)=35.80 p≤0.0001	F(1,50)=12.05 p= 0.0001	F(1,50)=96.32 p≤0.0001	F(1,50)=5.72 p=0.0205
LCAD	F(1,50)=323.8 p=0.0113	F(1,50)=222 p≤0.0001	F(1,50)=583.7 p≤0.0001	F(1,50)=28.07 p≤0.0001	F(1,50)=6.43 p= 0.0144	F(1,50)=79.80 p≤0.0001	F(1,50)=1.54 **p=0.2191, ns**
CD36	F(1,48)=1668 p≤0.0001	F(1,48)=189.1 p≤0.0001	F(1,48)=214.5 p≤0.0001	F(1,48)=20.82 p≤0.0001	F(1,48)=45.70 p≤0.0001	F(1,48)=1.20 **p= 0.2786, ns**	F(1,48)=28.90 p≤0.0001
LDL-R	F(1,50)=1204 p≤0.0001	F(1,50)=49.27 p≤0.0001	F(1,50)=1.07 **p= 0.3059, ns**	F(1,50)=63.49 p≤0.0001	F(1,50)=0.485 **p= 0.4891, ns**	F(1,50)=247.8 p≤0.0001	F(1,50)=297.3 p≤0.0001

Supplementary information to data in Figure 5. Summary of the effects of ageing, diet (AL or FR), OLLT and their interactions, obtained by three-way ANOVA followed by Tukey´s post hoc test, on the expression of liver genes involved in lipogenesis, fatty acid oxidation and glucose disposal. Statistical significance was set at p≤0.05.

Gene	OLLT	AGE	DIET	OLLT & AGE	OLLT & DIET	AGE & DIET	OLLT, AGE & DIET
FAS	F(1,50)=2034 p≤0.0001	F(1,50)=145.1 p≤0.0001	F(1,50)=0.839 **p= 0.3639, ns**	F(1,50)=122.0 p≤0.0001	F(1,50)=6.48 p= 0.0140	F(1,50)=162.6 p≤0.0001	F(1,50)=147.8 p≤0.0001
Scd-1	F(1,50)=626.9 p≤0.0001	F(1,50)=1903 p≤0.0001	F(1,50)=2427 p≤0.0001	F(1,50)=1751 p≤0.0001	F(1,50)=1401 p≤0.0001	F(1,50)=2096 p≤0.0001	F(1,50)=2297 p≤0.0001
ELOVL6	F(1,48)=2315 p≤0.0001	F(1,48)=65.35 p≤0.0001	F(1,48)=5.54 p=0.0227	F(1,48)=244.7 p≤0.0001	F(1,48)=23.27 p≤0.0001	F(1,48)=45.05 p≤0.0001	F(1,48)=184.9 p≤0.0001
DGAT2	F(1,48)=1161 p≤0.0001	F(1,48)=1173 p≤0.0001	F(1,48)=919 p≤0.0001	F(1,48)=340.4 p≤0.0001	F(1,48)=333.3 p≤0.0001	F(1,48)=1133 p≤0.0001	F(1,48)=559.7 p≤0.0001
MTTP	F(1,48)=8885 p≤0.0001	F(1,48)=114.1 p≤0.0001	F(1,48)=221.7 p≤0.0001	F(1,48)=48.06 p≤0.0001	F(1,48)=729.0 p≤0.0001	F(1,48)=226.8 p≤0.0001	F(1,48)=13.48 p≤0.0001
Pck1	F(1,50)=946.8 p≤0.0001	F(1,50)= 21.52 p≤0.0001	F(1,50)=30.79 p≤0.0001	F(1,50)=36.75 p≤0.0001	F(1,50)=24.76 p≤0.0001	F(1,50)=150.6 p≤0.0001	F(1,50)=6.02 p=0.0177
G6Pase	F(1,50)=319.9 p≤0.0001	F(1,50)=25.54 p≤0.0001	F(1,50)=4.20 p= 0.0456	F(1,50)=28.35 p≤0.0001	F(1,50)=7.66 p= 0.0079	F(1,50)=65.14 p≤0.0001	F(1,50)=65.20 p≤0.0001
GLUT2	F(1,50)=1062 p≤0.0001	F(1,50)=4.00 **p= 0.0509, ns**	F(1,50)=9.37 p= 0.0035	F(1,50)=4.38 p= 0.0413	F(1,50)=27.96 p≤0.0001	F(1,50)=1.47 **p= 0.2298, ns**	F(1,50)=51.51 p≤0.0001

Supplementary information to data in Figure 6. Summary of the effects of ageing, diet (AL or FR), OLLT and their interactions, obtained by three-way ANOVA followed by Tukey´s post hoc test, on TG content in liver and vWAT. Statistical significance was set at $p \leq 0.05$.

Tissue	OLLT	AGE	DIET	OLLT & AGE	OLLT & DIET	AGE & DIET	OLLT, AGE & DIET
Liver TG	F(1,30)=1.02 **p= 0.3207, ns**	F(1,30)=0.073 **p= 0.7883, ns**	F(1,30)=87.29 p≤0.0001	F(1,30)=1.58 **p= 0.218**	F(1,30)=25.71 p≤0.0001	F(1,30)=5.20 p= 0.0298	F(1,30)=13.68 p= 0.0009
vWAT TG	F(1,30)=244.2 p≤0.0001	F(1,30)=1.49 p= 0.2304	F(1,30)=59.54 p≤0.0001	F(1,30)=2.74 p= 0.1078	F(1,30)=4.84 p= 0.0353	F(1,30)=36.95 p≤0.0001	F(1,30)=5.78 p= 0.0223

Supplementary information to data in Figure 7. Summary of the effects of ageing, diet (AL or FR), OLLT and their interactions, obtained by three-way ANOVA followed by Tukey´s post hoc test, on the expression of vWAT genes involved in lipid disposal and glucose uptake. Statistical significance was set at $p \leq 0.05$.

Gene	OLLT	AGE	DIET	OLLT & AGE	OLLT & DIET	AGE & DIET	OLLT, AGE & DIET
PPARγ	F(1,58)=674.8 p≤0.0001	F(1,58)=184.2 p≤0.0001	F(1,58)=7.47 p= 0.0083	F(1,58)=279.2 p≤0.0001	F(1,58)=36.77 p≤0.0001	F(1,58)=156.1 p≤0.0001	F(1,58)=448.6 p≤0.0001
LPL	F(1,58)=3172 p≤0.0001	F(1,58)=875.4 p≤0.0001	F(1,58)=439.0 p≤0.0001	F(1,58)=144.1 p≤0.0001	F(1,58)=371.9 p≤0.0001	F(1,58)=1509 p≤0.0001	F(1,58)=1454 p≤0.0001
LDL-R	F(1,58)=60.56 p≤0.0001	F(1,58)=750.6 p≤0.0001	F(1,58)=64.94 p≤0.0001	F(1,58)=80.25 p≤0.0001	F(1,58)=188.1 p≤0.0001	F(1,58)=164.8 p≤0.0001	F(1,58)=341.0 p≤0.0001
CD36	F(1,58)=2403 p≤0.0001	F(1,58)=297.5 p≤0.0001	F(1,58)=64.27 p≤0.0001	F(1,58)=69.87 p≤0.0001	F(1,58)=34.11 p≤0.0001	F(1,58)=531.7 p≤0.0001	F(1,58)=306.9 p≤0.0001
Plin-1	F(1,58)=184.1 p≤0.0001	F(1,58)=61.25 p≤0.0001	F(1,58)=18.52 p≤0.0001	F(1,58)=356.3 p≤0.0001	F(1,58)=17.73 p≤0.0001	F(1,58)=0.541 **p= 0.4646, ns**	F(1,58)=0.187 **p= 0.6663, ns**
AQP7	F(1,58)=2564 p≤0.0001	F(1,58)=7.38 p= 0.0087	F(1,58)=11.18 p= 0.0015	F(1,58)=141.2 p≤0.0001	F(1,58)=7.34 p= 0.0088	F(1,58)=425.7 p≤0.0001	F(1,58)=446.3 p≤0.0001
GLUT4	F(1,58)=6.09 p= 0.0165	F(1,58)=184.4 p≤0.0001	F(1,58)=64.90 p≤0.0001	F(1,58)=627.6 p≤0.0001	F(1,58)=6.98 p= 0.0106	F(1,58)=262.7 p≤0.0001	F(1,58)=622.5 p≤0.0001
GLUT1	F(1,58)=17.95 p≤0.0001	F(1,58)=874.4 p≤0.0001	F(1,58)=17.30 p≤0.0001	F(1,58)=1876 p≤0.0001	F(1,58)=185.5 p≤0.0001	F(1,58)=0.008 **p= 0.9257, ns**	F(1,58)=314.4 p≤0.0001

Supplementary information to data in Figure 8. Summary of the effects of ageing, diet (AL or FR), OLLT and their interactions, obtained by three-way ANOVA followed by Tukey´s post hoc test, on the expression of vWAT genes involved in lipid oxidation, browning and inflammation. Statistical significance was set at $p \leq 0.05$.

Gene	OLLT	AGE	DIET	OLLT & AGE	OLLT & DIET	AGE & DIET	OLLT, AGE & DIET
CPT-1b	F(1,58)=92.87 p≤0.0001	F(1,58)=433.3 p≤0.0001	F(1,58)=6.33 p= 0.0146	F(1,58)=25.51 p≤0.0001	F(1,58)=281.8 p≤0.0001	F(1,58)=0.247 **p= 0.6206, ns**	F(1,58)=154.4 p≤0.0001
MCAD	F(1,58)=3456 p≤0.0001	F(1,58)=437.4 p≤0.0001	F(1,58)=283.8 p≤0.0001	F(1,58)=118.8 p≤0.0001	F(1,58)=254.6 p≤0.0001	F(1,58)=897.9 p≤0.0001	F(1,58)=824.7 p≤0.0001
LCAD	F(1,58)=21.73 p≤0.0001	F(1,58)=210.1 p≤0.0001	F(1,58)=23.38 p≤0.0001	F(1,58)=775.5 p≤0.0001	F(1,58)=24.04 p≤0.0001	F(1,58)=9.25 p= 0.0350	F(1,58)=21.17 p≤0.0001
TNF-α	F(1,58)=426.8 p≤0.0001	F(1,58)=22.67 p≤0.0001	F(1,58)=387.1 p≤0.0001	F(1,58)=193.8 p≤0.0001	F(1,58)=401.2 p≤0.0001	F(1,58)=6.45 p= 0.0138	F(1,58)=10.09 p= 0.0024
PGC-1α	F(1,58)=93.88 p≤0.0001	F(1,58)=473.6 p≤0.0001	F(1,58)=60.48 p≤0.0001	F(1,58)=188.5 p≤0.0001	F(1,58)=1.15 **p= 0.2867, ns**	F(1,58)=6.09 p= 0.0165	F(1,58)=16.03 p= 0.0002
PRDM16	F(1,58)=225.4 p≤0.0001	F(1,58)=96.89 p≤0.0001	F(1,58)=9.49 p= 0.0032	F(1,58)=134.6 p≤0.0001	F(1,58)=9.10 p= 0.0038	F(1,58)=4.11 p= 0.0047	F(1,58)=4.45 p= 0.0039
Adrβ3	F(1,58)=4920 p≤0.0001	F(1,58)=796.0 p≤0.0001	F(1,58)=409.2 p≤0.0001	F(1,58)=1078 p≤0.0001	F(1,58)=404.8 p≤0.0001	F(1,58)=28.98 p≤0.0001	F(1,58)=27.13 p≤0.0001
UCP-1	F(1,58)=363.8 p≤0.0001	F(1,58)=343.7 p≤0.0001	F(1,58)=287.4 p≤0.0001	F(1,58)=360.8 p≤0.0001	F(1,58)=290.2 p≤0.0001	F(1,58)=289.6 p≤0.0001	F(1,58)=282.4 p≤0.0001

Supplementary information to data in Figure 9. Summary of the effects of ageing, diet (AL or FR), OLLT and their interactions, obtained by three-way ANOVA followed by Tukey´s post hoc test, on the expression of ChREBP isoforms and FGF21 in liver and vWAT. Statistical significance was set at $p \leq 0.05$.

Gene	OLLT	AGE	DIET	OLLT & AGE	OLLT & DIET	AGE & DIET	OLLT, AGE & DIET
Liver ChREBPα	F(1,37)=3662 p≤0.0001	F(1,37)=5.63 p= 0.0229	F(1,37)=118.0 p≤0.0001	F(1,37)=85.14 p≤0.0001	F(1,37)=63.71 p≤0.0001	F(1,37)=174.3 p≤0.0001	F(1,37)=2.89 **p= 0.0971, ns**
Liver ChREBPβ	F(1,45)=14072 p≤0.0001	F(1,45)=7706 p≤0.0001	F(1,45)=8369 p≤0.0001	F(1,45)=6453 p≤0.0001	F(1,45)=7124 p≤0.0001	F(1,45)=9231 p≤0.0001	F(1,45)=10159 p≤0.0001
vWAT ChREBPα	F(1,45)=488.2 p≤0.0001	F(1,45)=42.44 p≤0.0001	F(1,45)=54.64 p≤0.0001	F(1,45)=2.22 **p= 0.1430, ns**	F(1,45)=42.13 p≤0.0001	F(1,45)=32.00 p≤0.0001	F(1,45)=38.97 p≤0.0001
vWAT ChREBPβ	F(1,45)=1568 p≤0.0001	F(1,45)=1436 p≤0.0001	F(1,45)=27.97 p≤0.0001	F(1,45)=1503 p≤0.0001	F(1,45)=22.16 p≤0.0001	F(1,45)=24.84 p≤0.0001	F(1,45)=17.77 p≤0.0001
Liver FGF21	F(1,50) =1208 p≤0.0001	F(1,50) =0.001 **p= 0.9732, ns**	F(1,50) =566.0 p≤0.0001	F(1,50) =50.96 p≤0.0001	F(1,50) =2.42 **p= 0.1255, ns**	F(1,50) =200.9 p≤0.0001	F(1,50) =77.89 p≤0.0001
vWAT FGF21	F(1,58)=215.0 p≤0.0001	F(1,58)=102.0 p≤0.0001	F(1,58)=7.40 **p= 0.0086, ns**	F(1,58)=744.4 p≤0.0001	F(1,58)=0.08 **p= 0.7780, ns**	F(1,58)=174.8 p≤0.0001	F(1,58)=345.1 p≤0.0001

Supplementary information to data in Figure 10. Summary of the effects of ageing, diet (AL or FR), OLLT and their interactions, obtained by three-way ANOVA followed by Tukey´s post hoc test, on serum FGF21 levels. Statistical significance was set at $p \leq 0.05$.

Serum levels	OLLT	AGE	DIET	OLLT & AGE	OLLT & DIET	AGE & DIET	OLLT, AGE & DIET
FGF21	$F(1,26)=774.1$ $p \leq 0.0001$	$F(1,26)=2059$ $p \leq 0.0001$	$F(1,26)=1120$ $p \leq 0.0001$	$F(1,26)=1598$ $p \leq 0.0001$	$F(1,26)=1511$ $p \leq 0.0001$	$F(1,26)=1045$ $p \leq 0.0001$	$F(1,26)=1138$ $p \leq 0.0001$