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Perilipin 1 ablation in mice enhances lipid oxidation during exercise and does not impair exercise performance

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ABSTRACT

Perilipin 1 is involved in the control of adipose tissue triacylglycerol hydrolysis. Its ablation in mice decreases fat mass and induces a partial resistance to diet-induced and genetic obesity. However, the consequences of perilipin 1 invalidation on energy balance are not fully defined. Moreover, the impact of perilipin 1 ablation on exercise performance and on fatty acids mobilization and utilization during exercise has not been studied. We compared energy balance (food intake, energy expenditure, spontaneous physical activity) and response to exercise of *Plin1*$^{-/-}$ and wild-type mice receiving a chow diet. The *Plin1*$^{-/-}$ mice had less fat, comparable food intake, comparable or slightly decreased energy expenditure, and no change in spontaneous physical activity. Mean 24-hour respiratory quotient was slightly lower, suggesting enhanced fatty acid oxidation. Exercise performance (both acute and endurance) was not impaired. Changes in nonesterified fatty acid levels during exercise were comparable, showing that triacylglycerol mobilization was unimpaired. Oxygen consumption increased faster (both tests) and to higher values (acute exercise) in *Plin1*$^{-/-}$ mice. Respiratory quotient increased during both types of exercise in *Plin1*$^{-/-}$ and control mice, but less in *Plin1*$^{-/-}$ mice. These lower respiratory quotient values show that *Plin1*$^{-/-}$ mice rely more on fatty acid oxidation during exercise. This is probably related to an overexpression in liver and muscle of genes for fatty acids oxidation. Perilipin 1 ablation has limited consequences on energy balance. It does not impair exercise performance; fatty acids mobilization during exercise is not impaired, whereas their oxidation is enhanced.

1. Introduction

Perilipin is a member of the previously called PAT (for perilipin, ADRP, and TIP47) family of proteins [1] that comprises also S3-12 [2] and LSDP5 (also called *OXPAT*) [3]. These proteins have highly related N-terminal sequences and share affinity for intracellular neutral lipid storage droplets. This family was recently renamed the *PERILIPIN* family, with perilipin being perilipin 1, ADRP perilipin 2, TIP47 perilipin 3, S3-12 perilipin 4, and LSDP5 perilipin 5 [4]. Alternative splicing gives rise to at least 3 distinct perilipin 1 proteins, A, B, and C, with A being the predominant form in adipose tissue [5]. Perilipin 1A is the most abundant phosphoprotein surrounding lipid droplets in adipocytes and has an important role in the control of intracellular triacylglycerol (TAG) storage and lipolysis [6]. In the basal state, that is, in the absence of stimulation of lipolysis,

Author contributions: MB designed the study. All authors participated in the collection and analysis of data, with MB, DL, and FF being mainly involved in the in vivo experiments, and SN and NH in messenger RNA measurements. All authors participated in manuscript writing.

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unphosphorylated perilipin 1 proteins are mainly present on the surface of lipid droplets and inhibit the action of lipases such as hormone-sensitive lipase (HSL) on stored TAG [7]. When lipolysis is stimulated by β-adrenergic agents, the phosphorylation of perilipin 1 in response to protein kinase A activation facilitates the action of phosphorylated HSL and promotes lipolysis [8,9]. Perilipin 1 is also involved in the control of adipose tissue triacylglycerol lipase (ATGL) activity through its interaction with CGI-58 [10]. Perilipin 1 knockout mice provided important information on the role of perilipin 1. In vitro, isolated adipocytes from *Plin1*$^{-/-}$ mice have increased basal lipolysis and attenuated lipolytic response to β-adrenergic agents [11,12]. This decreased response to isoproterenol was also observed in vivo [11], whereas basal values of glycerol and nonesterified fatty acids (NEFAs) are usually normal [11,12]. *Plin1*$^{-/-}$ mice have been found to be resistant to diet (high-fat)-induced and genetic obesity [11]. This was ascribed to an increased energy expenditure (EE) [11] and to increased tissue β-oxidation [13,14]. These results suggested that the lower expression of adipose tissue perilipin 1 found in human obesity [15-17] (with the exception of the study of Kern et al [18]) could be an adapting mechanism aimed at limiting the development of obesity and that decreasing further perilipin 1 expression or inactivating its activity could be useful for the prevention or treatment of obesity. However, although it is clear that *Plin1*$^{-/-}$ mice have less adipose tissue and more lean (muscular) mass [11,12], the consequences of perilipin 1 ablation on energy balance (food intake and total energy expenditure [TEE]) and on evolution of body weight are still unclear. For example, Martinez-Botas et al [11] reported that *Plin1*$^{-/-}$ mice receiving a chow diet had an increased food intake, whereas Tansey et al [12] found no difference. Similarly, Tansey et al found no difference in the evolution of body weight between *Plin1*$^{-/-}$ and control mice receiving a high-fat diet [12], contrary to Martinez-Botas et al [11]. Moreover, an increase of TEE in *Plin1*$^{-/-}$ mice was reported [11] but was evident only during a high-fat diet; it was only marginal during a chow diet, and Tansey et al [12] found no difference (unless mice were placed at thermoneutrality). Lastly, it has been shown that perilipin 1 overexpression also protects mice against diet-induced obesity [19]. This prompted us to reexamine the consequences of perilipin 1 ablation on the evolution of food intake, TEE, and body weight. Lastly, it has been clearly shown that both HSL and ATGL deletions impair fatty acid mobilization during exercise as well as exercise performance itself in mice [20,21]. Because perilipin 1 is also involved in the regulation of lipolysis and fatty acid mobilization, particularly in response to β-adrenergic stimulation [11,12], we investigated the response of *Plin1*$^{-/-}$ mice to exercise (maximal running capacity and endurance).

2. Materials and methods

2.1. Animals

C57BL/6 mice were from Charles River (L'Arbresle, France). *Plin1*$^{-/-}$ mice were a gift from I Tabas (Columbia University, New York, NY). These mice are from the strain generated and described by Martinez-Botas et al [11] and are on a C57BL/6 background. All mice were housed in an animal facility with controlled temperature (21°C-23°C) and lighting (light on 7:00 AM, light off 7:00 PM) and had free access to standard chow and water. *Plin1*$^{-/-}$ and C57Bl/6 mice were crossed to obtain F1 generation, heterozygous for the mutation of perilipin 1. These F1 mice were crossed to obtain *Plin1*$^{-/-}$ mice and their control wild-type (C57BL/6) mice. Male mice of the F3 or F4 generation were used for the various experiments. These experiments were conducted in the core facility ANIPHY where the mice were housed in the same conditions as those described above. This study was approved by the Ethical Committee of the University Claude Bernard of Lyon.

2.2. Protocols

2.2.1. Body weight and EE

Body weight, energy intake (recording of food consumption during 5 consecutive days), and expenditure were measured in 2-, 3-, and 4-month-old control and *Plin1*$^{-/-}$ mice (n = 6 for each group). Total energy expenditure was measured by a 24-hour recording of respiratory gaseous exchanges (indirect calorimetry). For this recording, mice were placed at 4:00 PM in individual cages placed in a room with controlled temperature (22°C ± 1°C) and lighting (light on at 7:00 AM and off at 19:00 PM). These cages allowed free access to water and food and were ventilated with room air (0.60 L/min). After an overnight habituation period, measurements of O_2 consumption (VO_2) and CO_2 (VCO_2) production were started at 9:00 AM and continued during 24 hours (Oxymat 800; Bioseb, Vitrolles, France). Respiratory quotient (RQ) is the ratio of the volume of CO_2 produced to the volume of O_2 consumed. Because urinary nitrogen excretion was not measured, we did not calculate rates of protein, lipid, and carbohydrate oxidation. However, RQ is an indicator of the relative amounts of lipid and carbohydrate oxidized. Total EE (kcal/[d $kg^{0.75}$]) was calculated using a rearrangement of the Weir equation as TEE = 1.44 VO_2(3.815 + 1.23 RQ) [22,23].

2.2.2. Spontaneous physical activity

Infrared frames: Spontaneous activity of mice placed in individual cages with free access to food and water was recorded using infrared frames. One frame (lower) recorded horizontal movements (*x* and *y* position), and another (upper) recorded rearing. Horizontal movements were first recorded as counts (beam breaks per time unit). Thereafter, the length of the path followed by the mouse was calculated from the modifications with time of the x,y position (software Actitrack, Bioseb, Vitrolles, France). After a habituation period of 16 hours, data were recorded during 24 hours. Data were then analyzed as activity during 24 hours, during the 12-hour light-on and -off periods, and per periods of 10 minutes to establish activity profiles.

Activity wheels: Mice were placed in individual cages equipped with activity wheel (Bioseb) and with free access to food and water. After a 24-hour habituation period, activity was recorded during 24 hours. Data were analyzed as for infrared frames (running distance during 24 hours, during the light-on and -off periods, and per 10-minute time units to establish activity profiles).

2.2.3. *Metabolic response to imposed physical exercise*

Mice were exercised on motorized treadmills equipped for simultaneous determination of respiratory exchanges (Bioseb). Before the experiments, mice were acclimatized to the treadmills during a 3-day period with 10 minutes of rest and 10 minutes of running (17 cm/s, 0° inclination) each day. Two tests were performed, one for evaluation of maximal running capacity (MAX test) and one for endurance capacity (endurance test). After an initial rest period, the MAX test started with a fixed inclination of 15° and a 10.2-m/min belt speed. Speed was thereafter increased every 2 minutes to 14.4, 18.6, 23.8, 25.2, 27.6, 30, 32.4, 34.8, 37.2, and 42 m/min. The test was stopped at *exhaustion* (defined as the mouse remaining on the shock grill for more than 4 seconds) or after the final step at 42 m/min if the mouse completed the test. The endurance test started, after an initial basal period, at a fixed inclination of 10° and a 10.2-m/min belt speed for 30 minutes followed by 15 minutes at 12.6 m/min, 15 minutes at 15 m/min, 15 minutes at 17.4 m/min, 15 minutes at 19.8 m/min, and a final step of 90 minutes at 22.2 m/min. The test was stopped at exhaustion or once the final step was completed. The day of the test, food was removed at 8:00 AM; and the test was initiated at 1:00 PM. Blood was first sampled (tail nicking) for basal blood glucose (OneTouch Ultra, LifeScan France, Issy les Moulineaux, France) and plasma NEFA (enzymatic method) determination. Thereafter, respiratory exchanges were recorded first during a 15-minute basal period and then throughout the whole test (air flow, 800 mL/min). When the test was stopped, blood was quickly collected for glucose and NEFA determination (end of test) before recording of respiratory exchanges during a 15-minute period and a final (recovery) blood sampling. Plasma D-β-hydroxybutyrate (D-βOHB) level was also measured (enzymatic method) at the end of the tests. The VO_2 consumption, EE, and RQ were calculated using data collected during the final 3 minutes of the basal and recovery periods (for both tests), the final 30 seconds of each step for the MAX test, and the final 2 minutes of each step for the endurance test.

2.2.4. *Response to fasting*

Respiratory exchanges were first measured in 5 control and 5 *Plin1*$^{-/-}$ mice during a 24-hour period as stated above. Thereafter, food was removed (at 8:00 AM); and respiratory exchanges were recorded for an additional 24-hour period. Blood (tail nicking) was collected for blood glucose and plasma NEFA determination just before food withdrawal and 12 and 24 hours after.

2.2.5. *Tissues glycogen concentrations and messenger RNA levels*

Control and *Plin1*$^{-/-}$ mice was anesthetized (intraperitoneal pentobarbital) at 1:00 PM (food withdrawn at 8:00 AM). Gastrocnemius, tibialis anterior, and soleus were quickly removed and immediately frozen in liquid nitrogen. Thereafter, samples of liver were collected and frozen. These samples were used for determination of glycogen content by enzymatic method (Biovision, San Francisco, CA) according to the manufacturer's instructions and for measurement of messenger RNAs (mRNAs) corresponding to genes for fatty acids oxidation (liver or muscle carnitine-palmitoyl transferase 1 [CPT1], long- and medium-chain acyl–coenzyme A [CoA] dehydrogenase, 3-hydroxyacyl-CoA dehydrogenase, 3-ketoacyl-CoA thiolase B, cellular fatty acid uptake [FAT]) and to genes of other members of the perilipin family (perilipins 2, 3, and 5). For these measurements, total RNAs were purified using TRIZOL protocol (Invitrogen, Cergy-Pontoise, France) with the addition of a DNase treatment. Concentrations and purity were verified by measuring optical density at 230, 260, and 280 nm and integrity by agarose gel electrophoresis. Total RNA was reverse transcribed using Superscript II (Invitrogen) and random hexamers. Real-time polymerase chain reaction was performed in a MyIQ thermal cycler (Bio-Rad, Marnes La Coquette, France) using iQ SYBR green Supermix (Bio-Rad). All samples were run in duplicate along with dilutions of known amounts of target sequence for quantification of initial complementary DNA copies. Results are expressed as the target over 18S RNA concentration ratio (ng/μg). Primer sequences are shown in Supplemental Table 1.

2.3. *Statistics*

Data are shown as mean and SEM. Comparisons between control and *Plin1*$^{-/-}$ mice were performed by 2-tailed Student *t* test for nonpaired values for body weight, 24-hour EE and RQ, physical activity, glycogen, and mRNA values. Within-group and between-groups comparisons during the exercise tests were performed by 1-way and 2-way analysis of variance (ANOVA) respectively, followed by Newman-Keuls (1-way ANOVA) and Bonferroni (2-way ANOVA) tests, respectively, to locate the differences. For Bonferroni test, the Bonferroni correction for multiple comparisons was applied. $P < .05$ was considered as indicating a significant difference. Calculations were performed using either Excel 2003 (Microsoft, Redmond, WA) or GraphPad Prism 5.03 software (GraphPad, San Diego, CA).

3. Results

3.1. *Evolution of body weight and EE*

Body weight, 24-hour food intake and EE, as well as RQ were determined in 2-, 3-, and 4-month-old control and *Plin1*$^{-/-}$ mice. Body weights were comparable in 2-month (23.0 ± 0.8 vs 22.7 ± 0.7 g) but slightly higher in 3-month (27.0 ± 0.3 vs 25.3 ± 0.4 g, $P < .05$) and 4-month (29.6 ± 0.4 vs 27.7 ± 0.5 g, $P < .05$) -old *Plin1*$^{-/-}$ mice (Fig. 1). Fat mass and fat-free mass were not determined; but at sacrifice, *Plin1*$^{-/-}$ mice had as expected a clear decrease of fat deposits (epididymal fat: 114 ± 6 vs 561 ± 73 mg, $P < .01$; perirenal fat: 85 ± 3 vs 188 ± 48 mg, $P < .05$ in 3-month-old mice). Therefore, fat-free mass was clearly increased in *Plin1*$^{-/-}$ mice. Food intakes were comparable in 2-, 3-, and 4-month-old control and *Plin1*$^{-/-}$ mice (data not shown). Total EE was comparable in 2- and 4-month-old mice (Fig. 1). It was slightly lower in 3-month-old *Plin1*$^{-/-}$ mice (180.2 ± 4.5 vs 209.0 ± 7.5 kcal/[d kg$^{0.75}$] in controls, $P < .05$); however, values were comparable when expressed on a per-animal basis (12.01 ± 0.27 vs 12.98 ± 0.47 kcal/d). In all groups of mice, EE was higher at night than during the light-on period

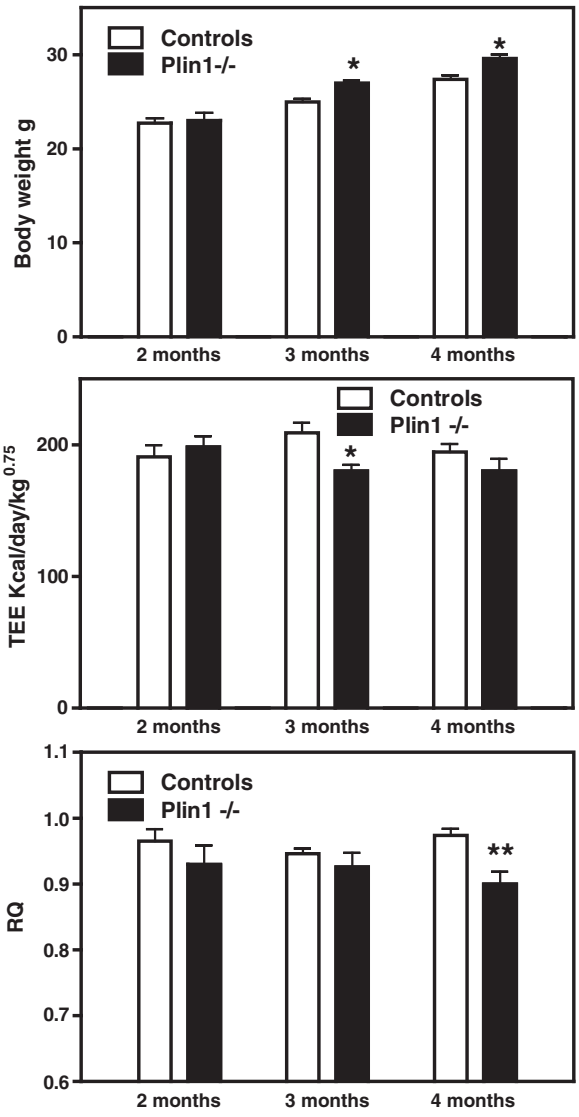

Fig. 1 – Body weights, TEE, and 24-hour mean RQ in 2-, 3-, and 4-month-old control and *Plin1*$^{-/-}$ mice (n = 6 per group). *P < .05, **P < .01 vs the corresponding control group (2-tailed Student *t* test for nonpaired values).

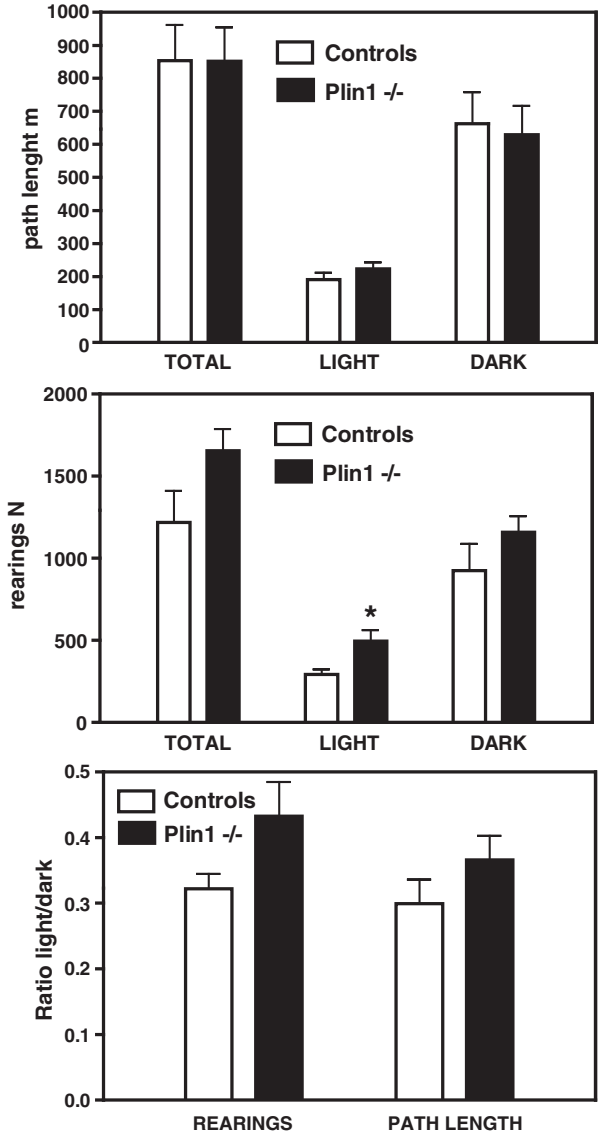

Fig. 2 – Spontaneous physical activity in 3-month-old control and *Plin1*$^{-/-}$ mice. Upper panel: length of the path traveled (total during 24 hours, and during the light and dark periods). Middle panel: number of rearings (total, light, and dark periods). Lower panel: light over dark periods activity ratios. *P < .05 vs control mice (2-tailed Student *t* test for nonpaired values).

(P < .05 for all groups). Overall, the mean 24-hour RQ was slightly lower in *Plin1*$^{-/-}$ mice' but the difference was significant (P < .01) only at 4 months. In all groups of mice, RQ values were higher (P < .01) in the dark than during the light-on period. The lower RQ in 4-month-old *Plin1*$^{-/-}$ mice was observed during both dark and light-on periods (P < .05 for both). Plasma NEFA values, determined in the fed state (at 8:00 AM), were comparable in *Plin1*$^{-/-}$ and control mice, even at 4 months (538 ± 65 vs 477 ± 32 μmol/L, not significant).

3.2. *Spontaneous physical activity*

We estimated 24-hour spontaneous physical activity first using infrared frames (n = 5) and then with activity wheels (n = 4) (3-month-old mice) (Fig. 2). Data obtained with the lower frame (locomotion) were used to calculate the length of the path followed by each mouse. No difference between *Plin1*$^{-/-}$ and control mice in 24-hour path length or in the repartition of path length between light and dark periods was found. Total rearing number was slightly increased in *Plin1*$^{-/-}$ mice; but the difference was significant only during the light period, and this did not significantly modify the repartition in rearing number between light and dark. When using activity wheels, we found no difference in running distance between control (3560 ± 396 m/24 h) and *Plin1*$^{-/-}$ mice (3385 ± 361 m/24 h); and the repartition of the activity was also identical with nearly all running (>90%) during the dark period. Overall, no significant difference in physical activity between *Plin1*$^{-/-}$ and control mice was detected.

3.3. *Treadmill exercises*

We performed 2 types of exercise, an estimation of maximal running capacity (MAX) and an endurance exercise. The changes in oxygen consumption and RQ during the MAX test are shown in Fig. 3. The number of mice completing a given step of the test is indicated in the upper panel of this figure. No significant difference in body weight was found between the control (27.2 ± 1.3 g) and *Plin1*$^{-/-}$ (27.0 ± 1.5 g) mice used in this experiment. All *Plin1*$^{-/-}$ mice stopped before the 32.4-m/min step and only 3 ran until the 30-m/min step, whereas 2 control mice ran until the 34.8-m/min step and one ran until the end of the 37.2-m/min step. However, there was no significant difference between the running distances (control: 246 ± 34 m, *Plin1*$^{-/-}$: 225 ± 20 m). Initial (basal) and final (during the recovery period) O_2 consumptions were identical. The VO_2 increased more and faster during the test in *Plin1*$^{-/-}$ mice (2-way ANOVA, genotype effect, $P < .01$) and was significantly higher than in control mice from step 3 to 6 in *Plin1*$^{-/-}$ mice. Moreover, the individual maximal values attained during the test by the mice for VO_2 and the calculated EE were higher in *Plin1*$^{-/-}$ mice (Student *t* test, $P < .01$, Fig. 3). Initial and final RQ values were slightly lower in *Plin1*$^{-/-}$ mice. Respiratory quotient increased as expected ($P < .01$) during the test in all mice; but the increase was more important in control mice, with final values near 1, than in *Plin1*$^{-/-}$ mice (2-way ANOVA, genotype effect, $P < .01$). In these mice, RQ values were significantly lower than in the control group during the third and fourth steps. In addition, the individual maximal RQ values attained were lower in *Plin1*$^{-/-}$ mice (Student *t* test, $P < .05$, Fig. 3). These results suggest a more important utilization of fatty acids during the test in *Plin1*$^{-/-}$ mice. This difference in RQ was observed despite near comparable evolutions in plasma NEFA and blood glucose concentrations (Table 1). Basal values were identical. The NEFAs were similarly increased at the end of the test and remained increased during recovery in both groups. However plasma D-βOHB concentrations were at the end of the tests higher in *Plin1*$^{-/-}$ mice (458 ± 46 vs 296 ± 31 μmol/L, $P < .05$, Student *t* test), showing that hepatic fatty acid oxidation was increased. Blood glucose increased during the test only in *Plin1*$^{-/-}$ mice and was increased during recovery in control and *Plin1*$^{-/-}$ mice.

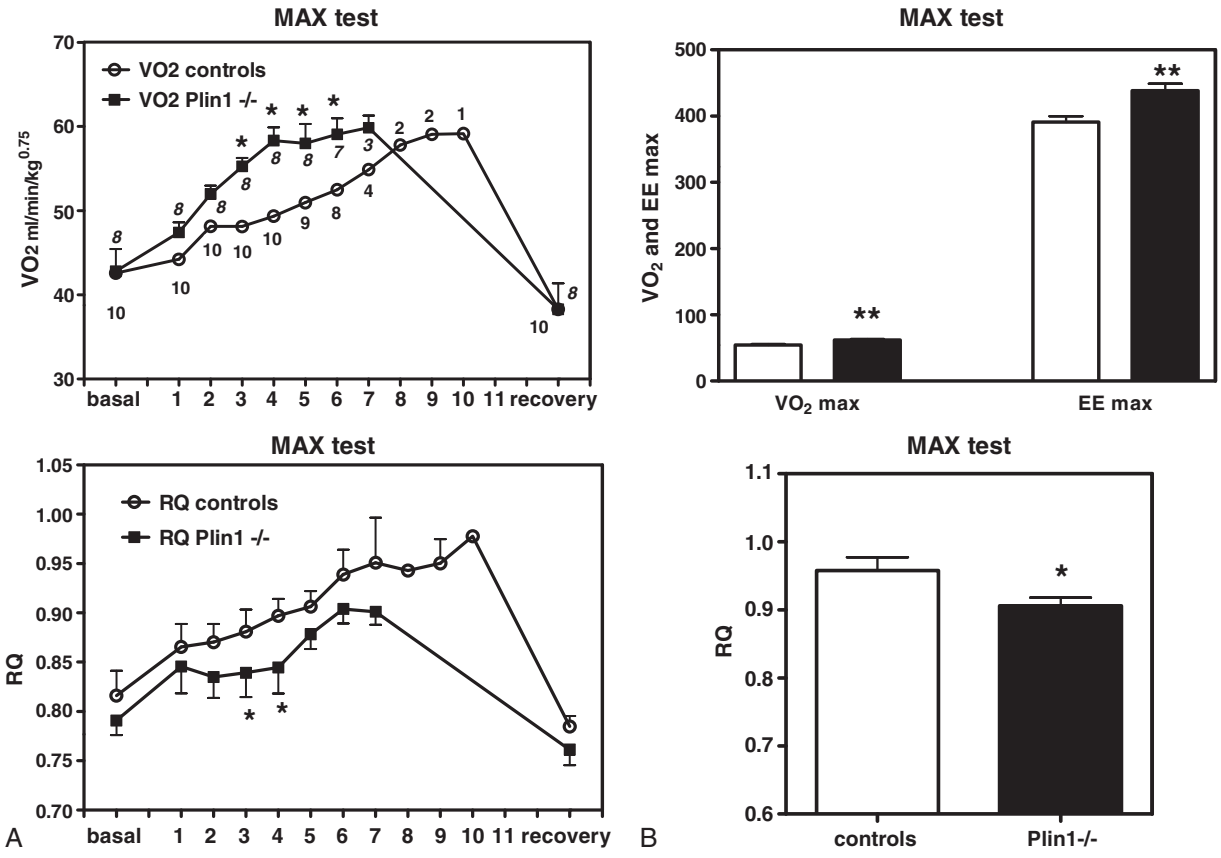

Fig. 3 – A, Oxygen consumption (upper panel) and RQ (lower panel) before (basal), during the successive steps (1 to 11 on the x-axis, referring to the successive belt speeds), and after the end (recovery) of the MAX test in control and *Plin1*$^{-/-}$ mice. The number of mice completing the various steps of the test is indicated in the upper panel. *P < .05, **P < .01 vs the corresponding step of the test in control mice (2-way ANOVA followed by Bonferroni test using Bonferroni correction for multiple comparisons). B, Comparison of the individual maximal values attained during the MAX test by control and *Plin1*$^{-/-}$ mice. Upper panel: oxygen consumption (mL/[min kg$^{0.75}$]) and EE (kcal/[d kg$^{0.75}$]); lower panel: RQ. *P < .05, **P < .01 vs control mice (2-tailed Student *t* test for nonpaired values).

Table 1 – Blood glucose and plasma NEFA levels during the MAX test

	Glucose (mg/100 mL)		NEFA (μmol/L)	
	Controls	*Plin1*$^{-/-}$	Controls	*Plin1*$^{-/-}$
Basal	162 ± 10	142 ± 9	583 ± 42	564 ± 57
End of test	167 ± 25	186 ± 14 *	707 ± 43 *	740 ± 114 *
Recovery	203 ± 26 *	201 ± 18 *	922 ± 10 †	788 ± 82 †

* P<.05 vs the basal values of the group (ANOVA followed by Newman-Keuls test).
† P<.01 vs the basal values of the group (ANOVA followed by Newman-Keuls test).

Fig. 4 shows the evolution of VO_2 and RQ during the endurance test. Control and *Plin1*$^{-/-}$ mice had comparable body weight (26.5 ± 0.5 g vs 27.3 ± 1.1 g in *Plin1*$^{-/-}$ mice). Only one *Plin1*$^{-/-}$ mouse ran until the last step of the test against 3 control mice. However, the running distances were not different (controls: 1120 ± 103 m, *Plin1*$^{-/-}$: 1028 ± 84 m). Initial and final (recovery) O_2 consumption and RQ were also comparable. Again, VO_2 increased sooner during the test in *Plin1*$^{-/-}$ mice and was significantly higher ($P < .05$) than in controls during the first and third steps. However, the individual maximal values of VO_2 and calculated EE attained were comparable in control and *Plin1*$^{-/-}$ mice (Fig. 4). Respiratory quotient increased moderately ($P < .05$) during the test in control but not in *Plin1*$^{-/-}$ mice, and values were significantly lower in this last group ($P < .05$) during the third and fourth steps of the test. The individual maximal RQ values attained were also lower in *Plin1*$^{-/-}$ mice ($P < .05$, Student *t* test), suggesting again a more important utilization of fatty acids during the endurance test in this group. Blood glucose and plasma NEFA levels were comparable in the basal state and had a similar evolution throughout the test: Nonesterified fatty acids were increased at the end of the running period and remained elevated during recovery, whereas glucose decreased progressively to reach significantly lower values during recovery (Table 2). Again, plasma D-βOHB concentrations were at the end of the tests higher in *Plin1*$^{-/-}$ mice (725 ± 168 vs 482 ± 109 μmol/L, $P < .05$, Student *t* test), suggesting an increase in liver fatty acids oxidation rate.

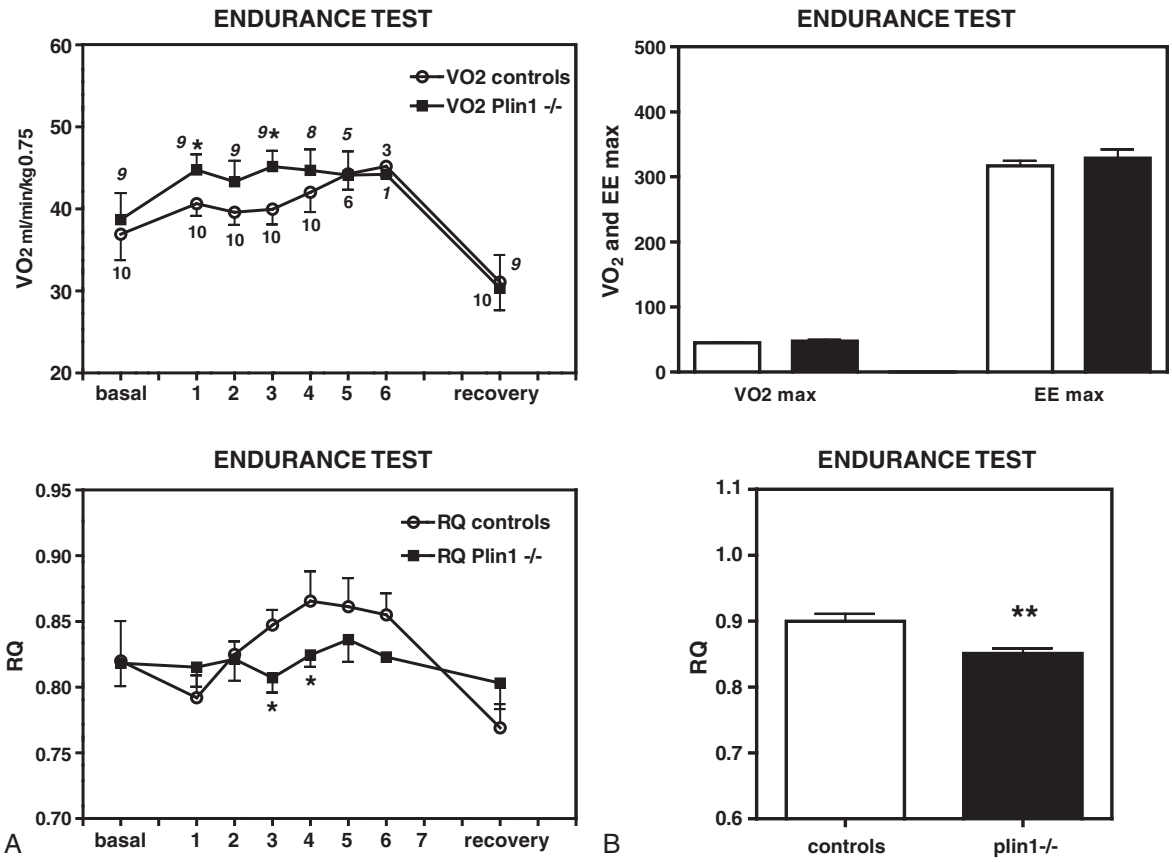

Fig. 4 – A, Oxygen consumption (upper panel) and RQ (lower panel) before (basal), during the successive steps (1 to 11 on the x-axis referring to the successive belt speeds), and after the end (recovery) of the endurance test in control and *Plin1*$^{-/-}$ mice. The number of mice completing the various steps of the test is indicated in the upper panel. *P < .05 vs the corresponding step of the test in control mice (2-way ANOVA followed by Bonferroni test with Bonferroni correction for multiple comparisons). B, Comparison of the individual maximal values attained during the endurance test by control and *Plin1*$^{-/-}$ mice. Upper panel: oxygen consumption (mL/[min kg$^{0.75}$]) and EE (kcal/ [d kg$^{0.75}$]); lower panel: RQ. **P < .01 vs control mice (2-tailed Student *t* test for nonpaired values).

Table 2 – Blood glucose and plasma NEFA levels during the endurance test

	Glucose (mg/100 mL)		NEFA (μmol/L)	
	Controls	*Plin1*$^{-/-}$	Controls	*Plin1*$^{-/-}$
Basal	173 ± 11	154 ± 7	648 ± 84	630 ± 65
End of test	138 ± 25	139 ± 21	940 ± 22 *	810 ± 101 *
Recovery	124 ± 21 *	117 ± 25 *	946 ± 8 *	779 ± 82 *

* $P < .05$ vs the basal values of the group (ANOVA followed by Newman-Keuls test).

3.4. *Response to a 24-hour fast*

Because we found differences in the evolution of RQ during exercise between control and *Plin*$^{-/-}$ mice, we checked the response of 3-month-old mice to another situation of stimulation of lipolysis and lipid oxidation, a 24-hour fast. During the 24-hour before food withdrawal, the RQ values in control and *Plin1*$^{-/-}$ mice were comparable to the values reported in the first paragraph of the results. Just before food withdrawal (at 8:00 AM), RQs were 0.86 ± 0.07 (controls) and 0.84 ± 0.04 (*Plin1*$^{-/-}$), NEFAs were 541 ± 102 and 459 ± 106 μmol/L, and blood glucose levels were 173 ± 29 and 153 ± 23 mg/100 mL. The evolution of these parameters during the fasting period was comparable (after 12 hours: 0.81 ± 0.01 vs 0.82 ± 0.02, 651 ± 135 vs 559 ± 93 μmol/L, and 165 ± 32 vs 147 ± 20 mg/100 mL; after 24 hours: 0.77 ± 0.02 vs 0.76 ± 0.03, 758 ± 101 vs 782 ± 81 μmol/L, and 132 ± 14 vs 121 ± 17 mg/100 mL).

3.5. *Tissues glycogen and mRNA concentrations*

These concentrations were determined in samples collected in the postabsorptive state, 6 hours after food withdrawal, that is, in the same conditions than for mice before starting treadmill experiments. Glycogen concentrations were comparable in control and *Plin1*$^{-/-}$ mice for liver (6.4 ± 1.6 vs 5.6 ± 1.6 μg/mg tissue) and gastrocnemius (0.53 ± 0.17 vs 0.42 ± 0.13) and moderately decreased in tibialis anterior (*Plin1*$^{-/-}$: 0.13 ± 0.07 vs 0.43 ± 0.15, $P = .09$). Messenger RNA values of genes for fatty acids oxidation are shown in Fig. 5. Medium-chain acyl-CoA dehydrogenase mRNA was largely increased in liver of *Plin1*$^{-/-}$ mice, with more moderate increases in CPT1, long-chain acyl-CoA dehydrogenase, and 3-hydroxyacyl-CoA dehydrogenase expressions. Expression of most of these genes was also moderately increased in soleus (a muscle with mainly oxidative fibers) and gastrocnemius (muscle with a mix of oxidative and glycolytic fibers) but not in tibialis anterior (a mainly glycolytic muscle). In addition, FAT mRNA values were higher in liver (+75% ± 21%, $P < .05$) and soleus (+54% ± 12%, $P < .05$) of *Plin1*$^{-/-}$ mice. Perilipins 2 and 3 mRNA levels were not significantly modified. However, perilipin 5 expression was increased in liver (+121% ± 32%, $P < .05$) and soleus (+175% ± 43%, $P < .05$).

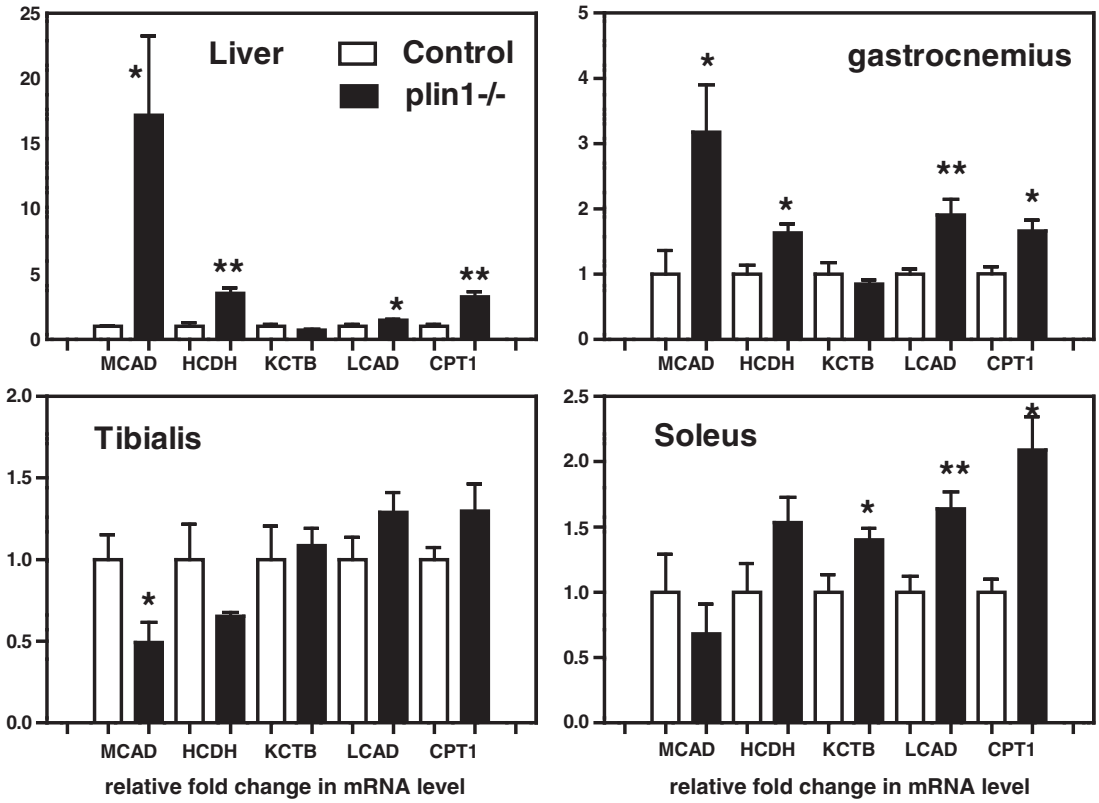

Fig. 5 – Expression (mRNA concentrations) of genes involved in fatty acids oxidation in liver and skeletal muscles of control and *Plin1*$^{-/-}$ mice. *$P < .05$, **$P < .01$ vs control mice (2-tailed Student *t* test for nonpaired values).

4. Discussion

We investigated in the present report the consequences of perilipin 1 invalidation in mice on energy balance (food intake and EE) and on exercise performance. This was performed in mice receiving a standard (chow) diet. In agreement with previous reports [11,12], we found that *Plin1*$^{-/-}$ mice have less fat than wild-type mice, particularly a large reduction in epididymal adipose tissue mass. This reduced fat mass was not accompanied by a decrease in body weight, which was unchanged or sometimes slightly increased. Although body composition was not directly measured, these data show, in agreement with previous studies [11,12], that *Plin1*$^{-/-}$ mice are leaner than wild-type, ones with decreased fat mass and increased fat-free mass. This higher fat-free mass was ascribed by Martinez-Botas et al [11] to a greater muscle mass. Contrary to these authors [11] but in agreement with Tansey et al [12], we found in *Plin1*$^{-/-}$ mice receiving a standard chow diet no increase in food intake or in EE. Food intake was unchanged, and EE was comparable or slightly decreased (in 3-month-old mice). Moreover, the discrepancy in EE between our data and those of Martinez-Botas et al is more apparent than real because (1) the increase in TEE these authors reported was evident only in mice receiving a high-fat diet and was very marginal when mice consumed a standard chow diet and (2) we and Martinez-Botas et al used different units for EE, and this could lead to apparently divergent results, as recently stressed [24,25]. Indeed, when using same units (VO_2 in mL/min on a per-animal basis), we found, as Martinez-Botas et al, no difference between control and *Plin1*$^{-/-}$ mice consuming a standard chow diet (3 months: 1.731 ± 0.043 vs 1.808 ± 0.075; 4 months: 1.707 ± 0.084 vs 1.792 ± 0.064). Therefore, we found overall no evident modifications of energy balance in *Plin1*$^{-/-}$ mice.

Spontaneous physical activity and activity profile were also unchanged, as estimated by infrared frames or when a more important activity level was established using activity wheels. Therefore, although *Plin1*$^{-/-}$ mice have less fat and more fat-free, probably muscular, mass, these modifications are observed in the absence of overall significant modifications of energy balance or spontaneous physical activity. An increase in lipolysis and in fatty acids oxidation could contribute to the decreased fat mass of *Plin1*$^{-/-}$ mice by promoting a simultaneous increase in TAG breakdown and fatty acids catabolism. In vitro, basal lipolysis of adipocytes isolated from *Plin1*$^{-/-}$ mice is enhanced [11,12]. In vivo, we found, in agreement with previous report [12], normal basal levels of plasma NEFA and a normal rise during a 24-hour fast. However, this finding of normal NEFA concentrations despite a reduced fat mass could be indicative of an enhanced lipolysis per unit of fat mass [14]. The expression of genes for fatty acids oxidation is increased in *Plin1*$^{-/-}$ mice [13,26], a finding we confirm, in both liver and skeletal muscles, in the strain used in the present report. In addition, the expression of perilipin 5, recently shown to be involved in the control of lipolysis and fatty acid oxidation in oxidative tissues [27] was increased. The in vitro oxidation of fatty acids by liver, muscle, and adipose tissue has also been found to be higher [14]. Our finding of a slightly lower RQ in *Plin1*$^{-/-}$ mice would support such an enhancement of lipid oxidation in vivo.

Deletion of HSL or ATGL impairs mobilization of TAG stores during exercise as shown by a reduced increase in plasma NEFA [20,21]. This occurs despite a normal increase in catecholamine [20]. Lipid oxidation during exercise is also impaired, whereas carbohydrate oxidation is increased [20,21]. These metabolic alterations are associated with impairment in exercise performance. Perilipin 1 ablation increases basal lipolysis, but the in vitro [11,12] and in vivo [14] lipolytic response to catecholamine is blunted. This last abnormality suggested that lipid mobilization and oxidation during exercise, and exercise performance, could also be impaired in *Plin1*$^{-/-}$ mice. However, exercise performance during either acute or endurance exercise was only marginally and not significantly impaired in these mice compared with wild-type mice. Mobilization of TAG stores, as appreciated by the evolution of plasma NEFA levels, was not impaired. This suggests that, although perilipin 1 has a clear role in the regulation of lipolysis, it has no rate-limiting role during exercise. It is also possible that compensatory changes in other lipid droplets associated proteins enable an adequate lipolytic response to exercise. Moreover, fatty acids oxidation during both acute and endurance exercise was moderately higher in *Plin1*$^{-/-}$ mice as shown by the lower RQ values attained. Therefore, the enhanced expression of genes for fatty acids oxidation previously described [13,26] and confirmed in the present report and the increased fatty acid oxidation rates by liver, muscle, and white adipose tissue of *Plin1*$^{-/-}$ mice described in vitro [14] result in vivo in an enhanced capacity to oxidize fatty acids during exercise. In addition, the moderate increases in FAT and perilipin 5 expression we observed may have contributed to the increase in fatty acid oxidation by promoting cellular fatty acid uptake and oxidation [27]. Lastly, a decrease in muscle glycogen stores and thus in carbohydrates availability, as found in the present report at least in tibialis anterior, could also have favored fatty acids oxidation during exercise. An unexpected finding is the greater increase in VO_2 during exercise in *Plin1*$^{-/-}$ mice. This could be explained in part by a more important muscle mass in these mice [12]. Another possibility is an increased dissipation of energy through uncoupling of oxidative phosphorylation because uncoupling proteins 2 and 3 (UCP2 and UCP3) expressions are clearly upregulated in *Plin1*$^{-/-}$ mice [14].

In conclusion, we found that perilipin 1 ablation induces no evident modifications in 24-hour energy balance and spontaneous physical activity, at least in mice fed a chow diet. During exercise, lipolysis is not impaired, whereas fatty acid oxidation is enhanced. Lastly, exercise performance is not significantly impaired in *Plin1*$^{-/-}$ mice. Although one has to be cautious when extrapolating to humans data obtained in mice, all the more that there are differences in the factors limiting exercise performance between mice and humans, these results suggest that the decreased expression of adipose tissue perilipin 1 found in human obesity [15-17] could limit the development of obesity by favoring fatty acids oxidation and should not impair exercise performance.

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Conflict of Interest

The authors have no conflict of interest to declare.

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