

Sensorimotor restriction during the early postnatal period affects rat somatic development and energy metabolism

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Keywords: developmental origin of health and disease, adipose tissue, organ weight, metabolic adaptation

Posted Date: October 15th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11192106/v1>

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Additional Declarations: No competing interests reported.

Abstract

Early-life physical activity is critical for optimal development and long-term health. While sensorimotor restriction (SMR) impacts muscle and brain development, its consequences on energy metabolism and growth of various organs remain unclear. This study investigates in rats the consequences of a SMR from birth to postnatal day 15 (P15) and P28 on body and organ growth, metabolic parameters. We show that SMR rats had lower body weight and fat mass overall. At P15, the organ weight of SMR rats was similar to that of control rats or higher (kidney, liver, thymus), suggesting a normal body development. With regard to muscle growth, the soleus muscle (a postural, slow-twitch muscle) showed atrophy as early as P15, while other hindlimb muscles (extensor digitorum longus, tibialis anterior) were affected later. At P28, white adipocytes had a lower surface area. Interscapular brown adipose tissue was more developed but its lipid content was decreased. The analysis of metabolic parameters showed a lower blood glucose level at both ages in SMR pups, and elevated lactate and ketone levels at P28, pointing to altered energy metabolism in the absence of a change in energy intake. Genes involved in thermogenesis and fatty acid metabolism were increased at P28. Overall, these data demonstrate that low physical activity during childhood not only affects skeletal muscle mass, but has also metabolic implications.

KEY POINTS

1. Early sensorimotor restriction (SMR) disrupts whole-body energy metabolism.
2. SMR lowers lipid content in brown and white adipose tissue.
3. SMR lowers blood glucose, raises lactate and ketones, especially by P28.
4. SMR increases brown fat mass and thermogenic gene expression by P28.
5. Soleus muscle atrophies by P15 while other hindlimb muscles are less affected.
6. Data highlight the key role of physical activity in regulating metabolism during development

INTRODUCTION

Adverse experiences during early stages of life can lead to a developmental programming, defined by Zambrano et al [1] as “the response to a specific challenge to the mammalian organism during a critical developmental time window that alters the trajectory of development with resulting effects on health that persist throughout life”, a concept called DOHaD (developmental origin of health and disease) [2, 3].

Rodents are altricials. They are very immature at birth and the immediate postnatal stage is a key period for the development of their physiological functions. Indeed, while the development of organs occurs during foetal life, the final processes of maturation are achieved during the lactation period. Thus, manipulating the postnatal environment in rat (through handling, maternal separation, undernutrition...)

can impede the optimal development of the organism and affect neurochemical, neuroendocrine and behavioural traits [2, 4]. The deleterious effects persist in the long term and can subsequently result to illness in later life. Early-life stress or the postnatal nutritional status are the most studied manipulation of postnatal environment. Surprisingly, the consequences of a sensorimotor restriction (SMR) in pups have received little attention. Yet, it is widely acknowledged that physical activity and interactions with the environment are particularly critical in the postnatal period. Low physical activity has been demonstrated to be a contributing factor to the development of metabolic disorders [5] and a low muscle mass and strength during childhood leads to detrimental effects on health [6–8].

In a rodent model of SMR, we and other have shown that diminished physical activity and low interactions with environment during the lactation period (from postnatal day 1 - P1 - to P28) strongly affects the somatic and motor development of pups. SMR pups exhibit a reduced body mass and display altered motor skills and memory, and some alterations (motor activity and motor skills, memory) persist over time, at least until P60 [9, 10]. These alterations are often interpreted as a consequence of muscle atrophy which affects mainly postural muscles such as the slow-twitch soleus, as a secondary outcome of sedentary lifestyle. They may also result from an impaired maturation of sensorimotor circuits. This has been evidenced by the delay in the acquisition of a mature posture and in the acquisition of primitive reflexes [11]. In fact, most studies conducted on the effect of early SMR have focused on the impact of early physical hypoactivity on the central nervous system or on the musculoskeletal system [10–15]. Besides, metabolic changes have also been observed in the soleus muscle, with a decrease in mitochondrial enzyme activities and a reduction in the mitochondrial respiration [16]. Such metabolic changes may impact not only skeletal muscle development but also growth of other organs. However, the consequences of SMR on energy metabolism have not been systematically investigated.

We hypothesized that early (from P1 to P28) SMR affects systemic energy metabolism in developing pups. The goal of the paper was to better characterize the short-term changes in energy metabolism associated with early SMR. To do so, we evaluated, at P15 and P28, whether SMR alters 1) adipose tissue composition and systemic metabolic parameters; 2) expression of genes involved in thermogenesis; 3) growth trajectories of multiple organs and muscles.

METHODS

Animals and experimental design

Experiments were conducted in accordance with the recommendation of the European Communities Council Directive 2010/63/UE. They received agreement of the Regional Committee on the Ethics of Animal Experiments (CEEA 75, reference number: APAFIS#2021-020818231865). All efforts were made to minimize the number of animals and their suffering.

CD-Sprague-Dawley rats were purchased from Charles River Laboratories (L'Arbresle, France). Animals were housed in conditions with constant humidity, temperature, and light (12:00 light, 12:00 obscurity),

with access to food and water ad libitum. At parturition (P1), litter size was adjusted to 10 pups per dam (5 males and 5 females when possible). Litters were then randomly assigned to the control (CTRL) or sensorimotor restriction (SMR) group. Body weight was assessed daily from P1 to P7, and then weekly.

We used a total of 147 CTRL (76 males and 71 females) and 100 SMR rats (48 males and 52 females), obtained from respectively 17 and 14 litters.

Sensorimotor restriction

In the SMR group, pups' hind limbs were immobilized in extended position from P1 to P28 for 16h/day (from 5 PM to 9 AM) as already described [11]. Briefly, a cast whose size was adapted to the pup growth was fixed with medical adhesive. As an indication, for pups at P15 and P28, whose body weight was near 33 g and 90 g respectively, the weight of the cast was 5 g and 10 g. The mother/pups separation did not exceed 15 min per day, and CTRL pups were also separated from the mother and handled in equal proportions.

Lactation test

Assessment of milk intake was performed on P12, i.e. around the lactation peak, in 79 CTRL and 53 SMR rats. At 10 a.m., the mother was removed from the housing cage and the pups were placed on a heated blanket to prevent hypothermia. After this 5-hour fasting period, the mother returned with the pups to allow re-feeding. The pups' body weight (BW) was measured just after mother removal and after a 1- and 2-h suckling period. The milk intake was estimated by calculating the difference of BW between renutrition and post-fasting values.

Body composition

Body composition was determined in 10 CTRL and 10 SMR rats. The same animals were tested at P12 and P21. The Minispec body composition analyzer LF90II (Bruker, Germany) measures the mass of fat, lean tissue and fluid, using a 6.2-MHz frequency pulse. The rat was placed in an acrylic cylinder (48-mm diameter) and was loosely restrained within the cylinder by pushing a plunger to restrict its. The cylinder was then positioned inside the bore of the magnet. The measurement took less than 2 min.

Blood sample and tissue removal

At P15 or P28, the blood glucose, ketone and lactate levels were determined from the tail blood. A drop of blood was obtained by tail snipping, then glucose, ketone and lactate were measured using automatic analyzers (Free Style Optium Neo, Accuchek, for glucose and ketone, Lactate scout 4, EKF diagnostic, for lactate). After that, animals were deeply anesthetized with isoflurane (induction 3% in 2 L/min air) followed by intraperitoneal administration of T61 (MSD Animal Health). Rats were exsanguinated with an

intracardiac perfusion of 0.9% ice-cold NaCl. Organs (kidneys, liver, spleen, adrenals, stomach and thymus) were removed and weighed. Subcutaneous retroperitoneal WAT and interscapular BAT (iBAT) depots were also dissected, weighed, and stored in a formalin solution at 4°C. Care was taken to separate the BAT from the surrounding WAT. Finally, soleus, EDL and tibialis anterior muscles were removed, weighed and stored at -80°C.

Histology

After fixation, adipose tissue was embedded in paraffin using automated stations (STP120, Leica and EG116, Leica), and thin sections (5 µm thick) were cut using a microtome (Leica RM2145). The sections were stained with hematoxylin and eosin using Autostainer XL (G40966H13, Leica). sWAT cell areas were calculated with Cellpose algorithm, with a minimum size set at 30 µm². More than 1500 adipocytes were quantified per animal (range: 1635–11355). Lipid content of iBAT was evaluated with ImageJ software on a region of interest of 1000 x 1000 µm. A threshold was set manually to separate lipid droplets from background and the total surface of lipid was expressed as a percentage of total area.

RNA extraction and quantification

Total RNA was extracted from 10 mg of snap-frozen brown adipose tissue (BAT) using the RNeasy Mini Kit (Qiagen, #74104), including on-column DNase digestion (RNase-Free DNase Set, Qiagen, #1023460). Briefly, tissues were weighed and mechanically lysed in 1 mL of TRIzol reagent (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA; #15596018) using a Raptor tissue homogenizer (Qiagen, Hilden, Germany). Following homogenization, 200 µL of chloroform (Carlo Erba Reagents, #412571) was added, and samples were immediately vortexed and centrifuged at 12,000 × g for 15 min at 4°C. The aqueous phase was collected, mixed with 1.5 volumes of 100% ethanol, and loaded onto RNeasy spin columns according to the manufacturer's instructions. After on-column DNase treatment, columns were washed, and total RNA was eluted in 30 µL of nuclease-free water. RNA concentration and purity were assessed using a NanoDrop Eight spectrophotometer (Thermo Scientific, Waltham, MA, USA) by measuring absorbance ratios at 260/280 and 260/230 nm.

RNA reverse transcription and quantitative PCR (RT-qPCR)

Reverse transcription was performed using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, #4368813) to synthesize cDNA from 1 µg of total RNA in a final reaction volume of 20 µL. Quantitative PCR (qPCR) was subsequently carried out using PowerTrack™ SYBR™ Green Master Mix (Applied Biosystems, #A46109). Prior to amplification, cDNA samples were diluted 1:10. Gene expression levels of target and housekeeping genes (see Table S1 for primer sequence) were analyzed using a QuantStudio™ 6 Pro Real-Time PCR System (Applied Biosystems). Relative mRNA expression was calculated using the 2^{-ΔΔCt} method, with normalization to the *Ppia* gene (coding for Cyclophilin A) as the reference gene.

Statistical analyses

Data are expressed as mean $\pm$ standard error (SEM). Normality and homogeneity of variances were evaluated by the Shapiro-Wilk test and *F* test, respectively. As no effect of sex was detected, male and female have been pooled. Body weight, muscle and organ weight and milk intake were tested by ANCOVA with the OrganScaleR software [17]. Body composition values were compared by two-way ANOVA with repeated measures followed by Sidak multiple comparison test. A *P* value of less than 0.05 was considered significant.

RESULTS

Effect of SMR on body composition

Body weight was 34.1 ± 0.7 g at P15 in CTRL rats. SMR rats displayed about 5% lower body weight than CTRL pups (*P* = 0.0680). The difference between CTRL and SMR was exacerbated at P28 (-13%, *P* < 0.0001).

To determine whether SMR affects body composition, we quantified fat tissue using a body composition analyser (Fig. 1). Specifically, the SMR group had a lower fat tissue percentage compared to the CTRL group (-34% and -15% at P12 and P21 respectively). It should also be noted that the percentage of fat tissue increased with age (+26% and +42% in CTRL and SMR rats respectively). Lean tissue was increased in SMR rats with respect to CTRL ones (+3% at both P12 and P15), as well as free body fluid (+14% and +6% at P12 and P21 respectively).

Analysis of adipose tissue

Fat or adipose tissue is a critical organ that regulates body energy metabolism. It is classified as white (WAT) and brown adipose tissue (BAT) with different characteristics and functions. WAT is the main organ of energy storage while BAT is a thermogenic organ with a particular importance during childhood. To determine whether the fat decrease concerned WAT or BAT or both, samples of subcutaneous retroperitoneal WAT and of interscapular BAT were collected for histological analysis.

Retroperitoneal sWAT was more abundant in SMR rats than in CTRL ones at P15 (+34%, *P* = 0.011). In contrast, the weight was lower at P28 (-45%, *P* = 0.0005). Histological analysis revealed that sWAT consisted exclusively of unilocular adipocytes, with no evidence of multilocular ("beige"/"brite") adipocytes in either CTRL or SMR rats (Fig. 2a). The adipocyte area in the sWAT of SMR subjects were found to be similar to those of the CTRL subjects at P15 (-11%, *P* = 0.4607, Tukey's post-hoc test) but smaller at P28 (-55%, *P* < 0.0001) (Fig. 2c). It should also be noted that adipocyte area did not vary from P15 to P28 in SMR rats (-13%, *P* = 0.4261) whereas an almost twofold increase was reported in CTRL animals (+74%, *P* < 0.0001). Thus, SMR results in a decrease in energy storage at P28.

We also removed and weighed iBAT. The weight of iBAT tended to be higher in SMR rats at P15 than in CTRL ones (+ 16%, $P = 0.0688$) and the increase was more pronounced at P28 (+ 32%, $P = 0.0011$). iBAT histology was analysed to determine the percentage of lipid droplets (Fig. 2bd). At P15, the brown adipocytes contained several small lipid droplets accounting for $9.5 \pm 2.5\%$ of the surface area in CTRL animals and $7.1 \pm 2.0\%$ in SMR ones ($P = 0.8769$, Tukey's multiple comparisons test). At P28, the lipid content was greatly increased in CTRL rats with respect to P15 ($27.4 \pm 2.7\%$, $P < 0.0001$) but was unchanged in SMR rats ($3.9 \pm 1.8\%$, $P = 0.7621$). Thus, the iBAT appeared to be much denser in SMR rats than in CTRL ones at P28 (CTRL vs SMR: $P < 0.0001$).

Effect of SMR on acute milk intake

The decrease in fat mass observed in SMR pups can be attributed to a decrease in food intake and/or to an increase in energy expenditure. Thus, to determine whether the lower body weight and energy storage are due to reduced feeding during lactation, we estimated milk intake during a 1-h and 2-h suckling periods that followed a 5-h fast at P12 (Fig. 3). After 1h of suckling, food intake was lower in SMR rats than in CTRL ones (-17%, $P = 0.0016$). In contrast, no difference was detected after 2h (-6%, $p = 0.0874$). These data suggest that milk intake was similar in both groups.

Effect of SMR on circulating metabolic parameters

We next evaluated whether systemic metabolism would be altered by SMR. We monitored metabolic pathways through glycemia, ketonemia and lactatemia in the morning (8:30 – 10:30 AM) (Fig. 4). Blood glucose was lower in SMR rats at both P15 (-7%, $P = 0.001$) and P28 (-5%, $P = 0.0043$).

Concerning the analysis of lactate level, the value was unchanged at P15 (+ 12%, $P = 0.4270$) but was higher in SMR group than CTRL group at P28 (+ 23%, $P = 0.0011$). In addition, values did vary according to age, with higher values at P28 than at P15, but only for SMR rats ($P = 0.0045$, Mann-Whitney).

The blood level of β -hydroxybutyrate (β -OHB) was unchanged in SMR rats compared with CTRL at P15 (+ 3%, $P = 0.7585$). However, the value was higher in SMR rats at P28 (+ 18%, $P = 0.0425$). In addition, blood level of β -FOHB was lower at P28 than P15 for both groups ($P < 0.0001$).

Effect of SMR on thermogenic gene expression

We previously shown that, concomitant to altered glucose metabolism, iBAT mass was increased while lipid content was decreased. We thus hypothesized that SMR alters thermogenic capacity. To test this, we evaluated mRNA levels of several genes involved in thermogenesis and fatty acid metabolism (*Ucp1*, *Dio2*, *Cox7a1*, *Prdm16*, *Fdnc5*, *Cidea*, *Ppara*, *Ppargc1a* and *Adrb3*) (Fig. 5). At P15, mRNA levels were unchanged in SMR rats. In contrast, at P28, all evaluated markers were increased, with the exception of *Cidea*, *Ppargc1a* and *Adrb3*. Hence, the combination of increased iBAT mass, reduced lipid droplet area, and upregulated thermogenic gene expression at P28 suggest a shift toward a more thermogenically active BAT.

Effect of SMR on muscle and organ growth

To determine whether metabolic changes were accompanied by an impaired growth of body organs, the weight of several peripheral organs was measured (Fig. 6, Table S3). At P15, the majority of organs of SMR rats presented a higher weight than CTRL (+ 6% to + 25%, $P < 0.05$, for kidneys, thymus, liver, stomach and spleen). This result suggests that rat's growth was increased from birth to P15. In contrast, at P28, lower weights were observed in SMR rats for the thymus (-11%, $P = 0.0198$) and the stomach (-12%, $P = 0.0079$). Thus, organ growth was impaired between P15 and P28 in SMR rats.

The weight of three hind limb muscles was also measured, as previous studies have provided evidence for a muscle atrophy in response to SMR [10, 18]. As expected, SMR induced a decrease in muscle weight (Fig. 6 and Table S3). At P15, SMR impacted exclusively the postural soleus muscle (SOL). The muscle weight was reduced by 32% (estimated marginal mean, $P < 0.001$) while the two other hind limb muscles (extensor digitorum longus – EDL – and tibialis anterior – TA) were unchanged (+ 7% and - 1% respectively). In contrast, at P28, the three muscles showed a lower weight ($P < 0.001$). The severity of atrophy was considerably more pronounced in the SOL (-31%) than in the EDL (-11%) or the TA (-16%). Interestingly, SMR-induced atrophy occurred earlier in the SOL than in the EDL and TA, with maximum atrophy already achieved by P15 (Fig. 6).

DISCUSSION

The objective of this study was to investigate the effects of early SMR on energy and metabolic homeostasis and on the mass of various organs at P15 and P28. The present study has demonstrated that SRM rats exhibited a reduced fat mass and a decrease in lipid content of white and brown adipose tissue, while glycemia levels decreased at P15 and P28 and ketonemia levels were elevated at P28. In addition, the study demonstrated that SMR exerts an influence on somatic development. Indeed, body weight was lower at both P15 and P28 in SRM rats, and this observation was accompanied by a delay in the growth of several organs at P28 and by a SOL atrophy as soon as P15. Whether these changes result from a lower milk intake and/or to hypothermia is discussed.

A lower body weight was observed in SMR rats compared to CTRL rats at both P15 and P28. Such a reduction in body weight has already been described in rats subjected to short (30 min) periods of immobilisation at P7-P13 or at P15-P20 [19], showing that repeated immobilisation, either of brief (30 min/day) or long duration (16 h/day) can durably affect body development. The lower body weight was accompanied by a lower fat mass, and to a lesser extent of lean mass. Muscle atrophy can participate to the decrease in absolute lean mass; however, its impact on body weight should be marginal. Notably, at P15, the organ weight of SMR rats was similar to that of CTRL rats or sometimes higher, suggesting a normal body development. In contrast, at P28, stomach and thymus exhibited a decrease in weight. With regard to muscle development, in contrast to the other organs, the SOL of RSM animals showed a sharp decrease in growth as soon as P15, while the subsequent evolution between P15 and P28 was then

similar for rats in both groups. This observation indicates that SOL atrophy may develop from the first postnatal days, likely as a consequence of the postural defect.

The decrease in fat mass observed in SMR pups can be attributed to a decrease in food intake and/or to an increase in energy expenditure. Motor and postural issues linked to hindlimb casting could impede accessing the nipples, resulting in a lower caloric intake for SMR pups. However, the present data regarding milk intake suggested that the pups were not undernourished and did not exhibit a lower caloric intake, at least at P12, although food intake it occurred mainly during the first hour when access to nipples was resumed in CTRL rats and was more evenly distributed over the two hours in SMR rats, maybe due to the smaller stomach. This result therefore needs to be considered carefully. Indeed, it is plausible that pups experienced a reduction in food intake during the night (immobilization period) and/or an augmentation in lactation between the removal of casts (8 am) and the beginning of the fasting period (10 am).

The decrease in iBAT mass and in sWAT adipocyte volume could also be indicative of an increase in energy expenditure. Indeed, WAT main function is energy storage from triglycerides and this tissue and show close functional interactions with skeletal muscle to supply fatty acids at times of high energy demand. Exercise increases BAT activity and its expansion in rodent models, although investigations provided some conflicting data [20], maybe due to the type of exercise [21]. Exercise training in adult mice increases expression of UCP1 (uncoupling protein 1), a marker of brown adipocytes, and brown-like adipocytes (called 'beige' or 'brite' adipocytes) are detected in the WAT, a phenomenon called 'browning' [22]. Cold exposure, like exercise, can induce an increase in BAT activity and browning of WAT [23], and higher levels of *UCP1* are generally observed in response to exposition to severe cold [24, 25]. Despite the fact that the SMR model is regarded as a model of hypoactivity, and thus should induce a lower energy expenditure, the cast weight may necessitate a greater quantity of energy for the pup to move. However, individual motor activity and metabolic expenditure are difficult to evaluate in pups during the suckling period. In the same way, the position of the littermate in the nest is known to strongly influences temperature [26], and thus potential hypothermia resulting from difficulties of pups to remain tightly packed in the nest with the dam during the casting episodes could also increase energy expenditure. Browning of WAT in response to exercise is known to be mediated by the binding of irisin, a myokine, to the surface of adipocytes [27]. Unexpectedly, our team has recently shown that plasmatic irisin level was higher in SMR rats than in control ones from P8 to P28 [11]. We also reported an increase in *Ucp1* mRNA levels and other markers of thermogenesis at P28. However, we did not detect any sign of « beige-ing » of sWAT. In addition, no difference in central temperature was noticed between CTRL and SMR rats (unpublished results), indicating that maintenance of body temperature was correctly achieved, likely due to BAT activation.

We provided evidence of hypoglycaemia in SMR rats at both ages, while ketonuria or lactate levels increased at P28. In addition, we showed that lactate and ketone levels varied with age (increase and decrease respectively). BAT activation in SMR rats could participate to the hypoglycaemia at both P15 and P28. Indeed, BAT generates heat by glucose and lipid oxidation and BAT activation, induced by

exercise for instance, is associated to glucose uptake in the BAT [23]. Another hypothesis is that malnutrition may cause a decrease in glycaemia. Supporting this, a study showed that severe malnutrition (leading to a 50% weight loss) during the suckling period caused a decrease in glycaemia [28]. However, the food intake of our pups was not affected by SMR although this observation should be treated with caution as mentioned above. This is further supported by the observation that β -OHB, which is produced in response to fasting and/or to intense exercise [28], exhibited lower levels in SMR rats.

Lactate level was increased in SMR rats which may reflect increased anaerobic glycolysis. Interestingly, our team has provided evidence for a reduction in mitochondrial respiration (decrease in mitochondrial enzyme activities mainly in the SOL and decrease in citrate synthase activity which reflects mitochondrial abundance) in SMR muscles [16]. This observation suggests that metabolism may be more dependent on glycolytic pathways in SMR rats and thus could explain the higher lactate level in SMR rats. Beside its metabolic function, lactate is considered as an exerkine. Indeed, in adult rat, the blood lactate concentration is higher after swimming exercise than at rest [29]. Unexpectedly, we have previously demonstrated that irisin level, which is also considered as an exerkine, was increased in SMR animals, suggesting an adaptive mechanism [18]. Thus, it cannot be excluded that lactate level may change in the same way as irisin level.

In conclusion, we give evidence that early SMR extends beyond localized muscle atrophy to disrupt global energy metabolism. We hypothesized that by prioritizing fuel utilization over storage, SMR compromises organ growth and fat deposition. Our results highlight the critical role of physical activity in regulating metabolism in pediatric populations.

Declarations

AUTHOR CONTRIBUTIONS

Conceptualization: Erwan Dupont, Marie-Hélène Canu, Delphine Eberlé; Methodology: Erwan Dupont, Marie-Hélène Canu, Delphine Eberlé; Formal analysis and investigation: Mélanie Van Gaever, Orlane Dupuis, Julien Girardie, Laetitia Cochon, Emilie Cadart, Lucas Buttin ; Resources: Laetitia Cochon, Julien Delezie; Writing - original draft preparation: Mélanie Van Gaever, Marie-Hélène Canu; Writing - review and editing: Julien Delezie, Mélanie Van Gaever, Delphine Eberlé, Erwan Dupont, Marie-Hélène Canu; Funding acquisition and supervision: Erwan Dupont, Marie-Hélène Canu.

Competing interests: The authors declare no competing interests.

Experiments were conducted in accordance with the recommendation of the European Communities Council Directive 2010/63/UE. They received agreement of the Regional Committee on the Ethics of Animal Experiments (CEEA 75, reference number: APAFIS#2021-020818231865). All efforts were made to minimize the number of animals and their suffering.

ACKNOWLEDGEMENTS

Experiments were performed in the Eurasport facility of Lille University.

FUNDING

M.V-G., O.D. and J.G. received a grant from the Hauts de France regional council.

Clinical trial number: not applicable

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Figures

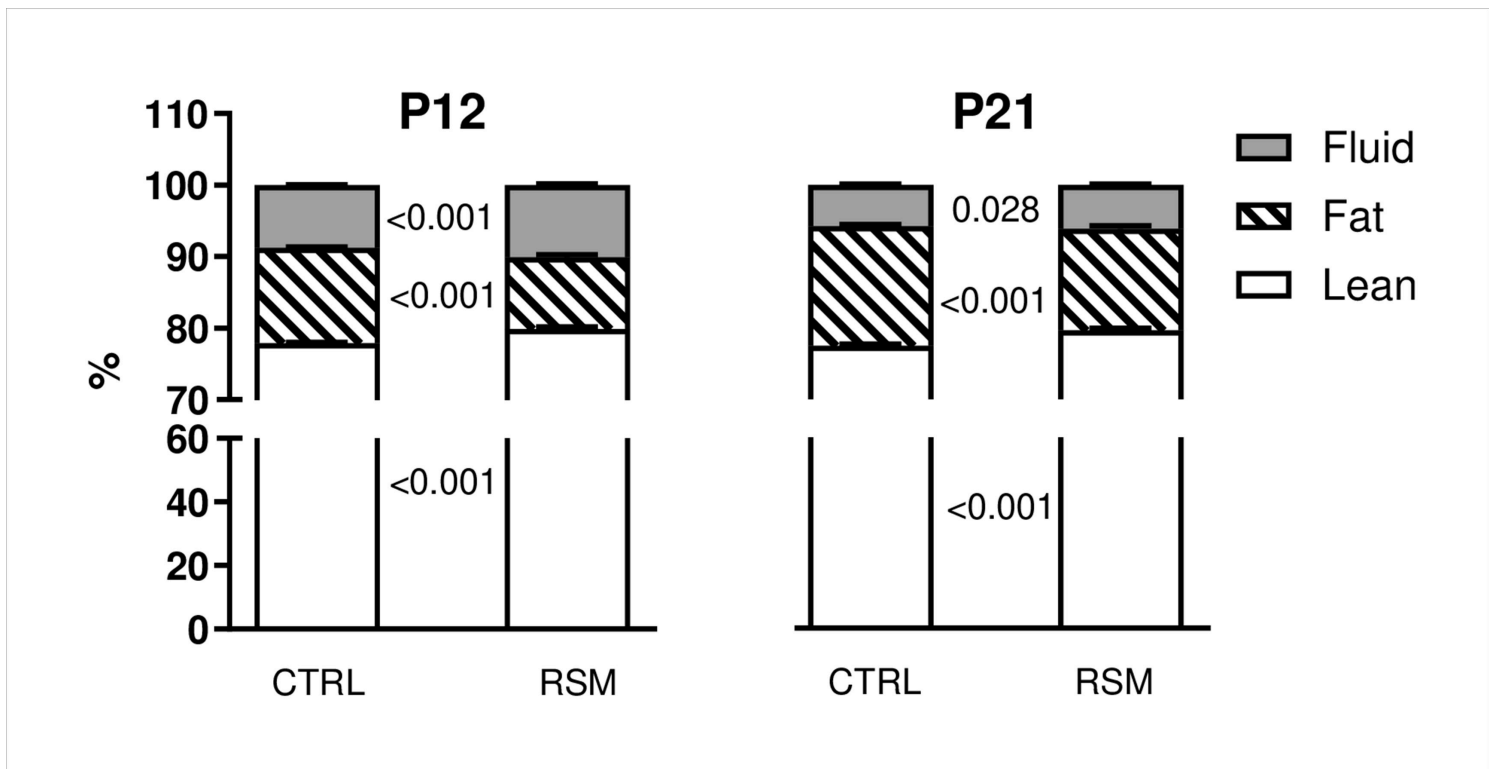


Figure 1

Body composition at P12 and P21. The proportion of fat mass, lean mass and body fluids was determined using a Minispec analyzer (Bruker) in 1 CTRL litter (n=10) and 1 SMR litter (n=10). Data were analyzed with two-way ANOVA with repeated measures followed by Sidak post-hoc test. F values for Group and Age effect and interaction are available in Table S2. Data are means $\pm$ SEM.

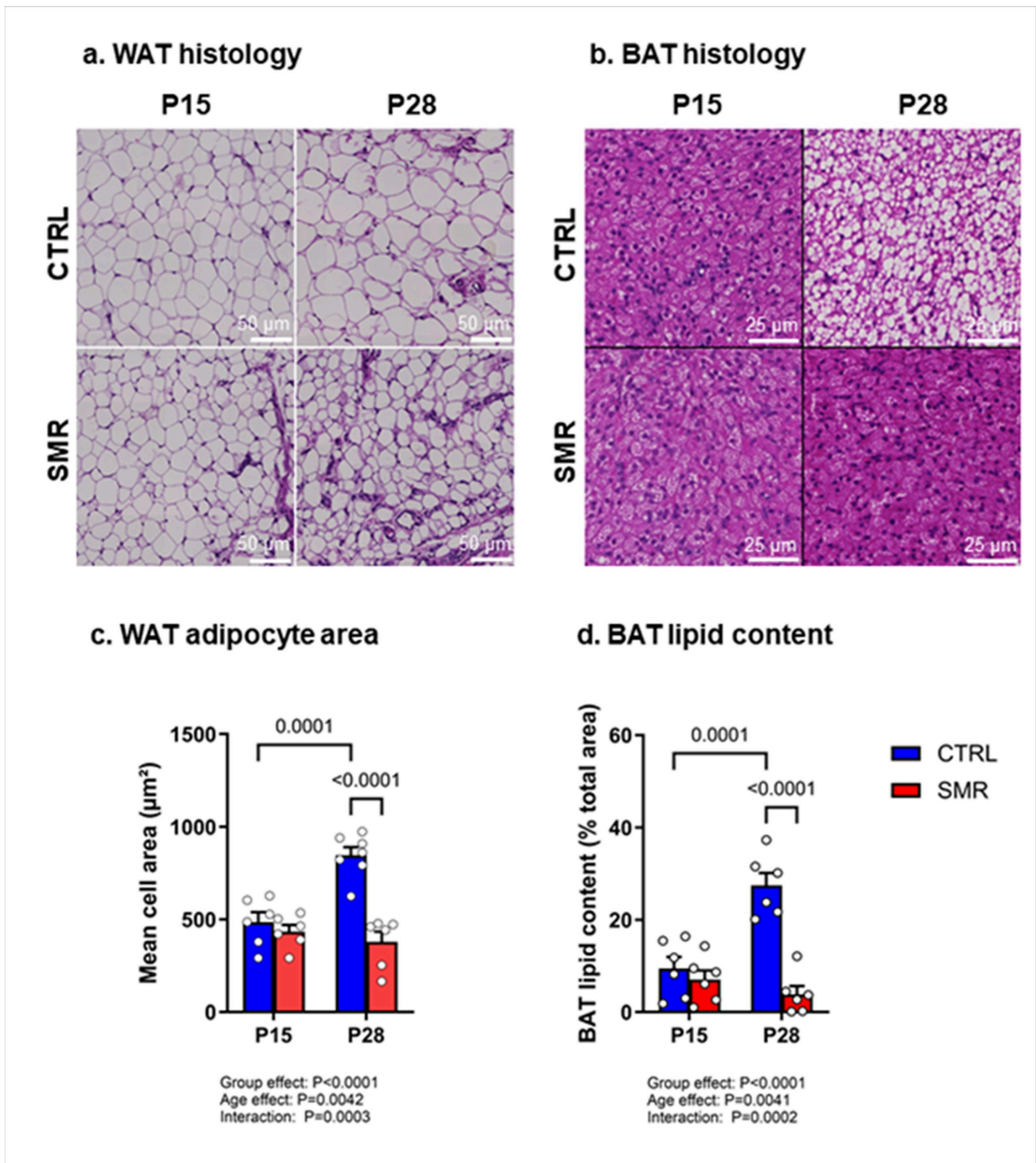


Figure 2

Analysis of white and brown adipose tissue. (a) Representative images of sWAT from CTRL and SMR pups, at P15 and P28. (b) Representative images of hematoxylin and eosin staining in iBAT. (c) Morphometric analysis showing distribution of adipocyte surface area. (d) Quantification of the lipid content within the iBAT. Individual points are median for one animal. Data were analysed with 2-ANOVA

with Tukey post-hoc test. CTRL P15: n=6 (from 2 litters); CTRL P28: n=6 (3); SMR P15: n=6 (3); SMR P28: n=6 (2).

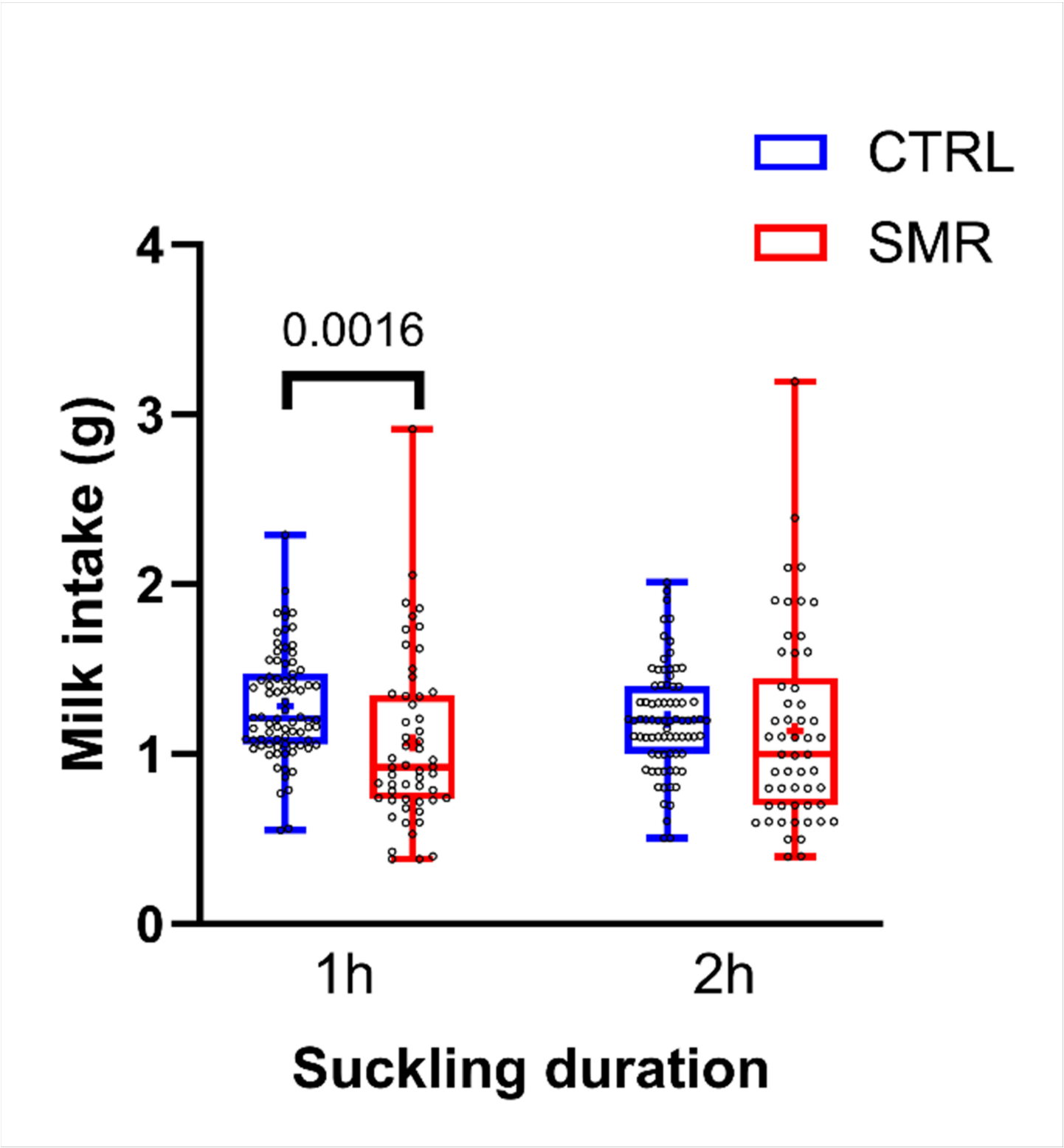


Figure 3

Milk intake. Milk intake was estimated in pups (P12) by comparing the body weight after a 5-hour-fasting and a subsequent 1h or 2h suckling period. Values are estimated marginal mean [95% CI]. Box

and whiskers show respectively median, 25% and 75% percentiles, and min / max values. The mean is indicated by +. Values were compared with ANCOVA. CTRL: n=79 (from 8 litters); SMR n=53 (6).

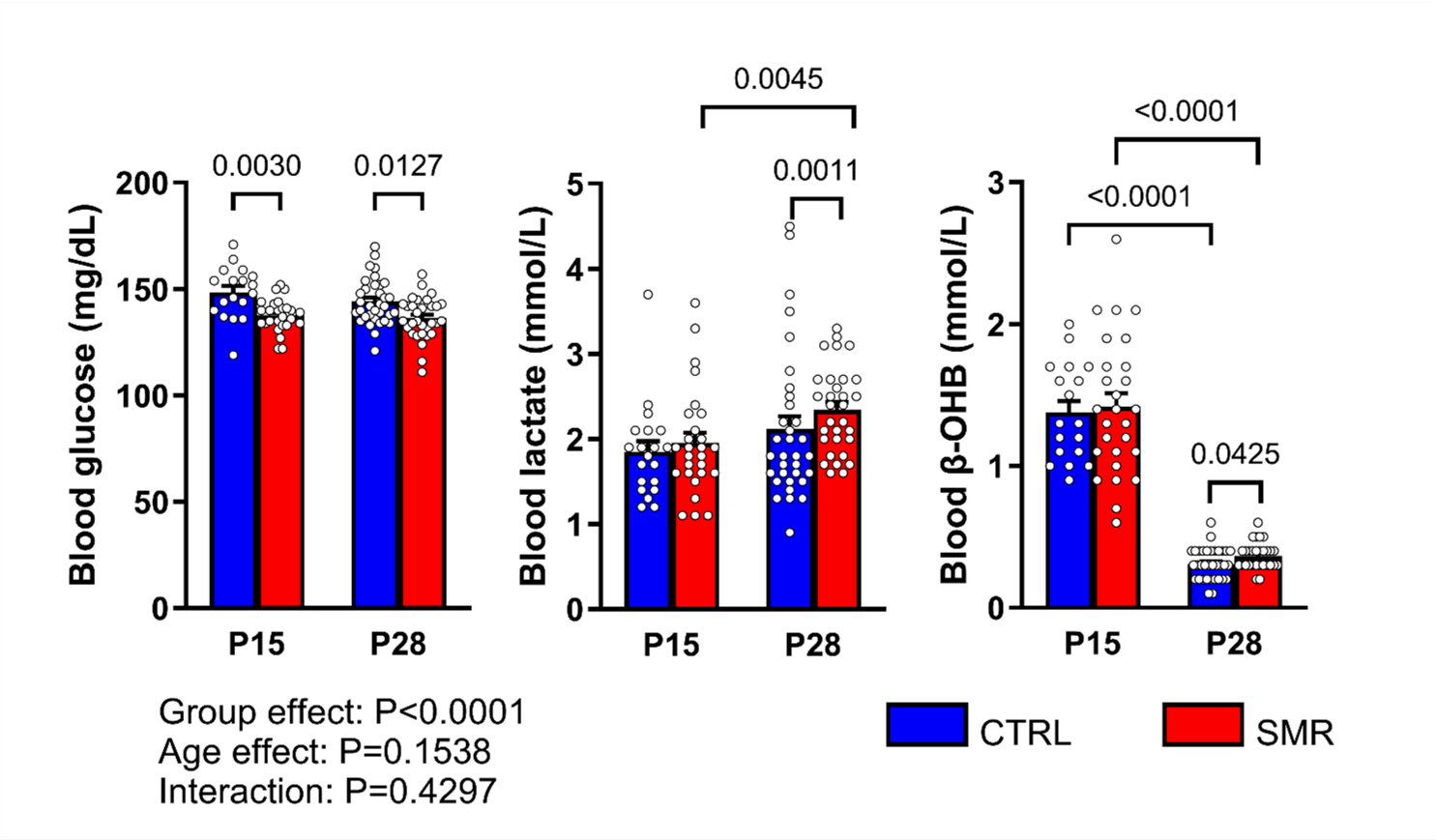


Figure 4

Metabolic parameters. Values are mean $\pm$ SEM. Data from blood glucose were compared by 2 way-Anova, with main effect of Group (CTRL vs. SMR) and Age (P15 vs. P28), followed by Sidak test. Ketonemia and lactatemia were analyzed by Mann-Whitney test. Data were obtained from 18 CTRL (from 2 litters) and 27 (3) SMR rats at P15, and 34 (4) CTRL and 30 (4) SMR rats at P28.

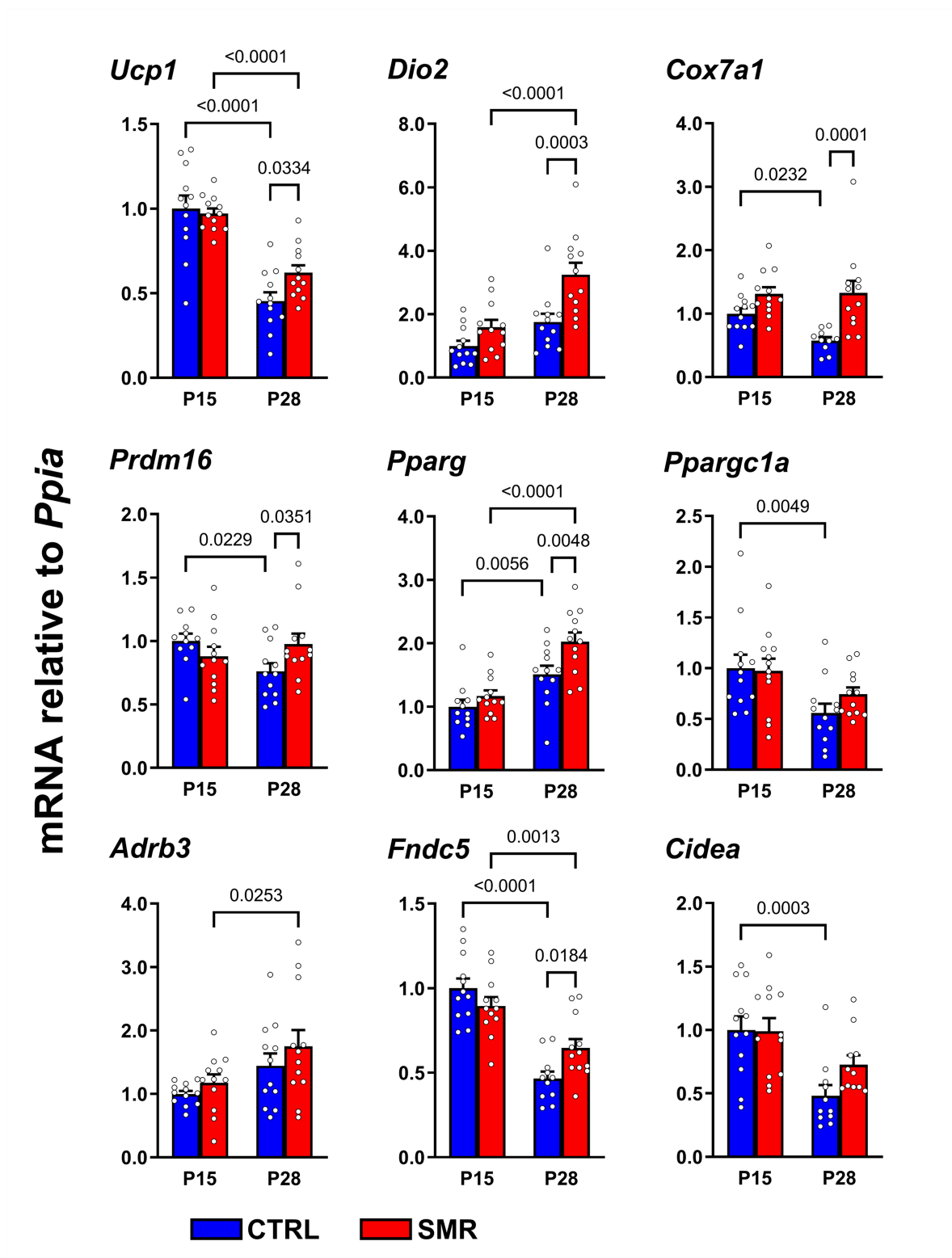


Figure 5

mRNA levels of thermogenesis markers in iBAT. Values are mean $\pm$ SEM. Data were compared with 2-way ANOVA followed by Fisher's LSD post-hoc test. F values for Group and Age effects are available in Table S2.

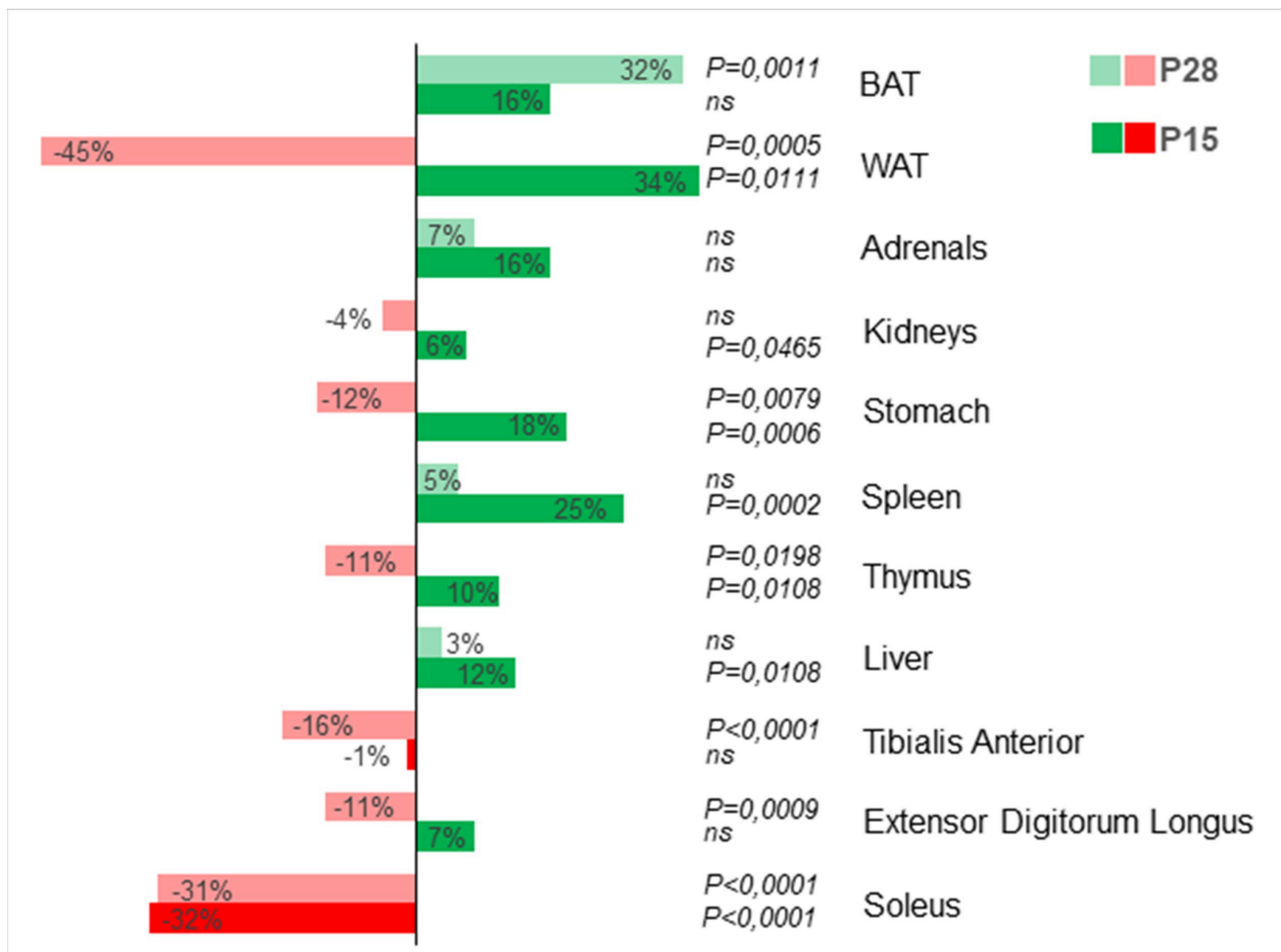


Figure 6

Muscle and organ weight at P15 and P28. Green and red bars indicate the percentage of decrease or increase in RSM rats relative to CTRL at P15 or P28. Data were compared with ANCOVA. Data were obtained from 22 CTRL (from 4 litters) and 31 (6) SMR rats at P15, and 35 (4) CTRL and 31 (4) SMR rats at P28.

Supplementary Files

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