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Abstract *Background and aims:* Maternal obesity and post-natal over-nutrition play an important role in programming glucose and lipid metabolism later in life. The aim of this study was to decipher the contributions of maternal obesity and post-natal over-nutrition on glucose and lipid metabolism in skeletal muscle.

Method and results: Male offspring of Sprague Dawley rat mothers fed either chow or high fat diet (HFD) for 5 weeks prior to mating were subsequently fed either chow or HFD until 18 weeks of age. Collection of plasma and skeletal muscle was performed at weaning (20 days) and 18 weeks. At weaning, offspring from obese mothers showed increased body weight, plasma insulin and lactate concentrations associated with reduced skeletal muscle glucose transporter 4 (GLUT4) and increased monocarboxylate transporter 1 (MCT1) protein. In 18-week old offspring, post-weaning HFD further exacerbated the elevated body weight caused by maternal obesity. Surprisingly this additive effect on body weight was not reflected in plasma glucose, insulin, lactate and MCT1; these markers were only increased by post-weaning HFD consumption. However, an additive effect of maternal obesity and post-weaning HFD led to decreased muscle GLUT4 levels, as well as mRNA levels of carnitine palmitoyl transferase-1, myogenic differentiation protein and myogenin.

Conclusion: Post-weaning HFD exerted an additive effect to that of maternal obesity on body weight and skeletal muscle markers of glucose and lipid metabolism but not on plasma glucose and insulin levels, suggesting that maternal obesity and post-natal over-nutrition impair skeletal muscle function *via* different mechanisms.

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Introduction

Metabolic abnormalities can have both genetic and environmental origins [26]. Regulatory mechanisms involved in food intake regulation and substrates (glucose/lipid) utilisation are highly plastic, especially early in life, and sensitive to adverse metabolic environments, resulting in life-long alterations, such as increased susceptibility for fat accumulation and glucose intolerance [11]. Intrauterine exposure to over-abundance of nutrients can result in negative metabolic programming, linking maternal obesity to childhood obesity [5]. Although the exact mechanisms associating maternal obesity to early-onset obesity or lipid/glucose metabolic dysfunction in offspring is still unclear, we and others have shown that "programming" of the neural circuitry involved in appetite regulation results in metabolic disorders in offspring [8,9,36]. Offspring from obese dams also show glucose intolerance and hepatic, pancreatic or muscle dysfunction [22,30,34], and post-natal nutritional environment can override genetic and prenatal factors, resulting in hyperphagia, insulin resistance and glucose intolerance [9,17].

Skeletal muscle, as the main site of insulin-stimulated and postprandial glucose disposal, is critical for glucose homeostasis [30], insulin resistance in skeletal muscle being strongly associated to metabolic disorders development [21]. Maternal obesity can adversely affect skeletal muscle development and its metabolic functions, offspring from obese rats showing reduced muscle contractile capacity, an energy-dependent function [3], probably in response to impaired insulin-signalling or mitochondrial capacity [2]. Post-natal over-nutrition can also negatively affect skeletal muscle metabolism. Rats fed a high fat diet (HFD) displayed impaired insulin-stimulated glucose uptake in skeletal muscle, altered insulin-signalling and intracellular lipid accumulation [4]. This negative "metabolic profile" was further confirmed by altered oxidative capacity in skeletal muscles in rats fed HFD [31] or by the hyperlipidemia and glucose intolerance observed in our offspring from obese dams, even when fed a low-fat diet [9]. Furthermore, an interaction between intrauterine over-nutrition and post-weaning HFD-consumption could exaggerate metabolic dysfunction [19,36].

A clear negative impact of both maternal obesity and post-natal over-nutrition has been observed on metabolic function, leading to glucose intolerance and insulin resistance at weaning and later in life. However, their interaction during these distinct developmental windows on skeletal muscle metabolism is still unclear. We hypothesized that maternal obesity and post-weaning over-nutrition have additive effects on skeletal muscle, leading to systemic metabolic dysfunction. Thus, this study aimed to investigate the impact of both maternal obesity and post-weaning HFD-consumption on fuel metabolism in skeletal muscles.

Methods

Animals

Protocols described here have been previously published and the animals used in this study were from the same cohort as those used in previous studies [8,9]. Twenty virgin outbred female Sprague Dawley rats (8-week old, Animal Resource Centre Pty.Ltd., Perth, Australia) were maintained on 12 h light/dark cycle, $20 \pm 1\,°C$, with 2–3 animals per cage. They were randomly assigned to a control group fed standard chow (11 kJ/g, 14% fat, 21% protein, 65% carbohydrates, Gordon's Speciality Stockfeeds, NSW, Australia) or a HFD group receiving a palatable cafeteria-style HFD (15.3 kJ/g, 34% fat, 19% protein, 47% carbohydrates), with similar starting body weight [8]. After 5 weeks of diet, females were mated with 10-week old male rats from the same source. During gestation, female rats were housed individually and monitored regularly. The same diet was maintained until weaning (post-natal day 20). The study was approved by the Ethics Committee of the University of New South Wales.

Post-natal dietary intervention

At birth, litter size was adjusted to 12 pups per mother (gender ratio: 1/1). At weaning, 8 male offspring from each maternal group (chow/HFD, Fig. 1) were anesthetised (ketamine/xylazine 180/32 mg/kg, i.p.). Blood samples were collected through cardiac puncture and rats were killed by decapitation. Plasma was stored for glucose, insulin and lactate determination. Soleus, extensor digitorum longus (EDL) and red tibialis anterior muscles were immediately dissected, weighed and snap frozen in liquid nitrogen and kept at $-80\,°C$ for mRNA, protein and enzyme activity quantification [24]. Fat pads (retroperitoneal, epididymal and mesenteric white adipose tissues) were collected and weighed. Half of the remaining male offspring from each litter were weaned onto chow or HFD, generating 4 post-weaning experimental groups with 7 rats per group (Fig. 1): chow-chow (CC), chow-HFD (CH), HFD-chow (HC) and HFD-HFD (HH). They were maintained on their respective diet until 18 weeks of age. After an overnight fast, they were anesthetised (ketamine/xylazine 180/32 mg/kg, i.p.) and blood samples were obtained by cardiac puncture before decapitation. Skeletal muscles and fat pads were collected and stored as described above.

Biochemical analysis

Plasma glucose and lactate levels were measured using an automated system (EML105-analyser, Radiometer Medical A/S, Copenhagen, Denmark). Insulin concentration was assessed using a radioimmunoassay kit (Linco, St.Charles, USA). The Homeostasis Model Assessment (HOMA) index was calculated to assess insulin resistance using the following formula: fasting plasma insulin (ng/ml) × fasting plasma glucose (mM)/(22.5 × 0.0417) [9]. Muscle triglyceride content was assessed in soleus muscle after total lipids extraction from muscle homogenates in 2:1 chloroform:methanol [14]. The organic phase was obtained after addition of NaCl and centrifugation and was air-dried overnight. Samples were reconstituted in absolute ethanol and assayed for triglycerides after enzymatic hydrolysis of triglycerides using a triglyceride reagent (Roche, NSW, AUS) and standards (Sigma, NSW, AUS).
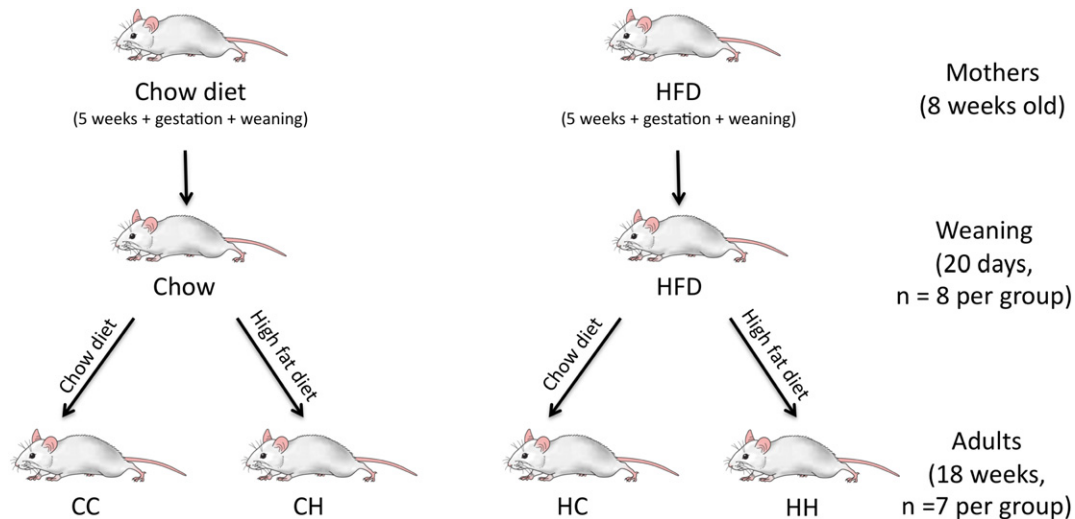
Figure 1 Experimental setting HFD: high fat diet, CC: mother on chow diet and post-weaning chow diet, CH: mother on chow diet and post-weaning HFD, HC: mother on HFD and post-weaning chow diet, HH: mother on HFD and post-weaning HFD.

Western blotting

In-house polyclonal antibodies against monocarboxylate transporters (MCT)1 and 4 were used [12]. Total proteins extracts were isolated from skeletal muscles (red tibialis anterior from weaning rats, soleus and EDL from adult rats) and protein concentration was assessed using the bicinchoninic acid assay (Pierce, Montlucon, France) [28]. Protein (40 μg) of muscle homogenates and prestained molecular mass markers (MagicMark, Invitrogen, Groningen, The Netherlands) were separated on 12% SDS-polyacrylamide gels (Novex System, Invitrogen, Groningen, The Netherlands) and transferred to polyvinylidene difluoride membranes which were then probed with primary antibodies (GLUT4, Santa Cruz Biotechnology, CA, USA; MCT1 or MCT4 antibody, 1:3000) and then with a anti-goat or anti-rabbit IgG horseradish peroxidase-conjugated secondary antibody (1:3000; BioSys, Compiegne, France). Protein expression was detected by enhanced chemiluminescence and the density of proteins bands was determined with Scion Image software (Frederick, MD, USA).

Enzyme activities

Supernatant from proteins extracts was assessed for citrate synthase (CS), 3-hydroxyacyl-coenzyme A dehydrogenase (HADH) and lactate dehydrogenase (LDH) activity. CS activity was measured with 0.38 mM oxaloacetate and 1.235 mM acetyl-CoA by recording the absorbance change at 412 nm over 2.5 min at 30 °C. HADH was measured with 0.17 mM reduced nicotanamide adenine dinucleotide (NADH) and 0.1 mM acetoacetyl-CoA, at 340 nm over 10 min at 25 °C. LDH activity was measured using 0.17 mM NADH and 2.5 mM sodium pyruvate at 340 nm over 2.5 min at 25 °C. Results are expressed as umol/min/g of tissue.

Quantitative real-time PCR

Total RNA was isolated from red tibialis anterior from weaning rats and soleus from 18-week old rats (30—100 mg) by homogenizing tissue in 1 ml TriZol reagent (Invitrogen, Melbourne, Australia). RNA was separated using chloroform and precipitated with isopropanol. Purified RNA (1 ng) was used as template to generate first-strand cDNA using random primer and Moloney Murine Leukemia Virus Reverse Transcriptase, ribonuclease H Minus, point mutant kit (Promega, Madison, USA). Preoptimized TaqMan probe/primers labelled with FAM (Applied Biosystems, Foster City, USA) were used for quantitative real-time PCR (Realplex 2; Eppendorf, Hamburg, Germany) on target genes (Peroxisome proliferator-activated receptor gamma coactivator-1 alpha or *Pgc1α*, myogenic differentiation protein or *myoD*, myogenin or *Myog* and carnitine palmitoyl transferase or *Cpt1*). Probes for the housekeeping gene (18s) were labelled with VIC. Target genes expression was quantified by single multiplexing reaction and normalised by the housekeeping gene and a sample from the control group was used as a calibrator.

Statistical analysis

Data are presented as mean ± SE. Body weight, systemic variables, enzymatic activity and protein and RNA levels were compared at weaning using Student's *t*-test. The difference between CC, CH, HC and HH was tested using factorial ANOVA and post-hoc analysis with Fisher's LSD test. Correlations were assessed using Pearson's test.

Results

As we previously reported, mothers fed HFD were more than 20% heavier before mating and after the birth and their energy intake before pregnancy was more than doubled compared to chow fed animals [9].

Impact of maternal obesity on male offspring at weaning

Weaning rats from obese mothers were heavier and had bigger skeletal muscle and fat mass than those from lean mothers ($p < 0.05$, Table 1), and the difference remained

Table 1 Anthropometry, circulating glucose, insulin, lactate and triglyceride levels and muscle enzyme activity.

	Chow	HFD	CC	CH	HC	HH
	(20 day-old)		(18-week-old)			
Body weight (g)	33.2 ± 0.3	47.4 ± 1.4^a	460 ± 9	$573 \pm 20^{d,f}$	506 ± 8^d	691 ± 25 [d,e,f]
Soleus (mg) [b,c]	10.3 ± 0.7	14.4 ± 0.6^a	183 ± 5	226 ± 18	212 ± 7	238 ± 8
EDL (mg) [b,c]	10.8 ± 0.8	15.4 ± 0.7^a	203 ± 6	232 ± 6	231 ± 8	246 ± 10
Tibialis (mg)	79.9 ± 1.9	99.0 ± 3.6^a	701 ± 28	738 ± 38	672 ± 23	744 ± 17
Fat mass [b,c] (g)	0.23 ± 0.01	0.65 ± 0.06^a	11.8 ± 0.6	37.7 ± 3.8	16.3 ± 0.9	64.7 ± 5.9
Glucose (mmol/l) [c]	7.9 ± 0.3	7.6 ± 0.1	8.6 ± 1.1	11.3 ± 0.7	6.9 ± 0.6	11.2 ± 0.7
Lactate (mmol/l) [c]	2.1 ± 0.2	2.7 ± 0.2^a	0.7 ± 0.1	1.1 ± 0.1	0.7 ± 0.0	1.0 ± 0.1
Insulin (ng/ml) [c]	0.22 ± 0.40	0.51 ± 0.06^a	0.10 ± 0.08	0.38 ± 0.04	0.12 ± 0.02	0.58 ± 0.10
HOMA [b,c] [9]			0.94 ± 0.19	4.56 ± 0.51	0.95 ± 0.22	7.30 ± 1.74
Triglycerides (ug/mg protein) [b]			75.0 ± 25.7	87.3 ± 42.7	131.4 ± 91.0	146.1 ± 79.5
	Tibialis		Soleus			
Citrate synthase (umol/min/g) [b]	67.7 ± 8.0	61.9 ± 5.2	30.8 ± 2.1^e	25.0 ± 1.4	29.7 ± 2.0	35.5 ± 0.9 [e,f]
Hydroxyacyl-Coenzyme A dehydrogenase	6.9 ± 0.4	7.9 ± 0.7	18.1 ± 1.7	15.5 ± 1.0	16.2 ± 0.7	22.4 ± 0.6 [d,e,f]
Lactate dehydrogenase (umol/min/g) [c]	402 ± 21	433 ± 37	86.8 ± 11.2	107.8 ± 9.8	85.8 ± 8.1	129.6 ± 11.1

Values are means $\pm$ SE. HFD: high fat diet, CC: mother on chow diet and post-weaning chow diet, CH: mother on chow diet and post-weaning HFD, HC: mother on HFD and post-weaning chow diet, HH: mother on HFD and post-weaning HFD, EDL: extensor digitorum longus, Fat mass: consists of retroperitoneal, epididymal and mesenteric white adipose tissues.
 [a] Significant difference with Chow, $p < 0.05$.
 [b] maternal effect, $p < 0.05$.
 [c] post-weaning HFD effect, $p < 0.05$.
 [d] significant difference with CC, $p < 0.05$.
 [e] significant difference with CH, $p < 0.05$.
 [f] significant difference with HC, $p < 0.05$.

even when normalised by body mass ($p < 0.05$, data not shown). Maternal obesity had no impact on glucose levels at weaning, but significantly increased insulin and lactate concentrations ($p < 0.05$, Table 1), both being positively correlated with body weight ($r = 0.78$, $p < 0.001$; $r = 0.43$, $p = 0.05$; $n = 16$, respectively).

Maternal obesity significantly decreased muscle GLUT4 at weaning ($p < 0.05$, Fig. 2) but increased MCT1 ($p < 0.05$, Fig. 2), and tended to increase MCT4, without reaching significance ($p = 0.09$, Fig. 2). GLUT4 was negatively correlated with body weight ($r = -0.60$, $p = 0.02$, $n = 14$), confirming its negative regulation by obesity [8]; however, GLUT4 was positively correlated with CS activity ($r = 0.73$, $p = 0.01$, $n = 14$). No effect of maternal obesity was observed on muscle enzymatic activity at weaning (Table 1). Rats from obese mothers showed a significant decrease in *myoD* levels, but only a trend for *Myog* ($p = 0.06$), *Pgc1α* ($p = 0.14$) and *Cpt1* levels ($p = 0.1$; Fig. 3).

Interaction between maternal obesity and post-weaning over-nutrition at 18 weeks

An interaction between maternal obesity and post-weaning HFD led to greater body weight (HH > CH > HC > CC, $p < 0.05$, Table 1). Both maternal obesity and post-weaning diet affected soleus and EDL muscle and fat pads weights, but had no impact on tibialis ($p < 0.05$, Table 1).

Circulating levels of glucose, insulin and lactate were all up-regulated by only post-weaning over-nutrition ($p < 0.05$, Table 1), and were positively correlated with body weight ($r = 0.45$, $p = 0.01$; $r = 0.62$, $p < 0.001$; $r = 0.49$, $p = 0.005$; $n = 32, 32, 31$ respectively). Both maternal obesity and post-weaning over-nutrition contributed to insulin resistance with a higher HOMA index in pups from obese dams and in those consuming HFD post-weaning ($p < 0.05$, Table 1). Maternal obesity, but not post-weaning over-nutrition, significantly increased triglyceride content in soleus muscle ($p < 0.05$, Table 1).

Maternal obesity and post-weaning over-nutrition decreased GLUT4 levels in oxidative muscle (soleus), GLUT4 being negatively correlated with body weight ($r = -0.65$, $p < 0.001$; $n = 28$). However, only post-weaning over-nutrition had an impact on glycolytic muscle (EDL; Fig. 2). Post-weaning over-nutrition significantly decreased MCT1 but only in soleus (Fig. 2).

Maternal obesity significantly increased the activity of both CS and HADH. An interaction between maternal obesity and post-weaning over-nutrition resulted in higher CS activity in CC versus CH and in HH versus CH and HC, and higher HADH activity in HH compared to the other 3 groups (Table 1). LDH activity was only increased by post-weaning diet (Table 1) and was positively correlated with body weight ($r = 0.68$, $p < 0.001$, $n = 28$) and insulin levels ($r = 0.62$, $p = 0.001$, $n = 28$).

Both maternal obesity and post-weaning over-nutrition decreased *myoD* and *Myog* expression (Fig. 3). *Cpt1* was decreased by post-weaning over-nutrition, which interacted with maternal obesity to further reduce its
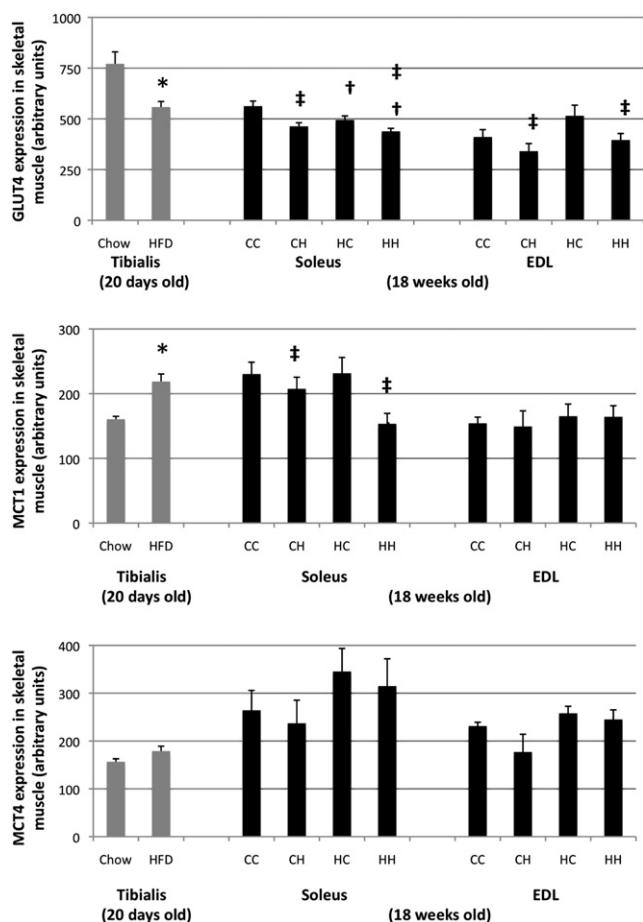
Figure 2 Glucose and monocarboxylate transporters in skeletal muscle. GLUT4: glucose transporter 4, MCT: monocarboxylate transporter, EDL: extensor digitorum longus, HFD: high fat diet, CC: mother on chow diet and post-weaning chow diet, CH: mother on chow diet and post-weaning HFD, HC: mother on HFD and post-weaning chow diet, HH: mother on HFD and post-weaning HFD, ∗ significant difference with Chow, $p < 0.05$; † maternal effect, $p < 0.05$; ‡ post-weaning HFD effect, $p < 0.05$.

expression in HH compared to CC and CH. *Pgc1α* expression was not influenced by either of the treatments.

Discussion

We demonstrate for the first time a negative impact of both maternal obesity and post-weaning over-nutrition on key markers of muscle metabolism contributing to insulin resistance. We also provide evidence for the additive negative impact of these two factors on muscle substrate metabolism. Most of the detrimental impact of maternal obesity was observed as early as weaning, but some beneficial adaptations were shown, mainly through increased MCT1 and fatty acid oxidative capacity at 18 weeks. These adaptations could potentially counteract some detrimental maternal impact, but were overridden by post-weaning HFD-consumption.

Our weaning rats from obese dams had increased body weight, fat mass, plasma insulin and lactate concentrations. The increased body weight observed at weaning in offspring from obese mothers has already been reported by our laboratory [8,9] but differs from other studies [2,19,29]. Although

the mechanisms responsible for the discrepancy in these results are still unclear, it can be postulated that the difference in animal strains, initial body weight of dams before diet and at mating, litter size during lactation, and diet composition used could all contribute. Also, the richer milk composition reported in obese mothers [17], could represent a contributing mechanism for the greater body weight in offspring from obese mothers [8]. We have previously reported that weight gain due to maternal obesity or post-natal over-nutrition mainly resulted from fat accumulation [8,9]. Increased adiposity can enhance insulin secretion and supports the hyperinsulinemia observed in our weaning and adult obese rats [1,33]. Hyperinsulinemia seemed to prevent hyperglycaemia at weaning, but failed to do so in adult rats consuming HFD, supporting insulin resistance from weaning age. Post-weaning over-nutrition could be a more powerful factor than maternal obesity on fat accumulation and insulin resistance as we have previously suggested [9], exaggerating the detrimental impact of the latter.

As the main site of insulin-stimulated glucose disposal, through the translocation of GLUT4 to the plasma membrane [27], skeletal muscle is critical for glucose homeostasis [30], and reduced GLUT4 levels are associated with insulin resistance and diabetes [21]. Here we report decreased GLUT4 in response to both maternal obesity and post-weaning over-nutrition. Several mechanisms have been linked to impaired GLUT4 levels and insulin resistance in skeletal muscles, but the accumulation of intramuscular lipids, potentially due to mitochondrial defects, represents one of the main candidates [20,32]. In weaning rats, no significant difference was observed in CS or HADH activity, suggesting a preserved oxidative capacity. In adults, an increased activity of both enzymes was observed in response to maternal obesity. In response to increased fat mass and circulating levels of triglycerides, lipid uptake is increased in skeletal muscles, leading to an up-regulation of lipid oxidation (HADH activity). Fatty acid oxidation is entirely dependent on oxidative capacity, which can be altered by intramuscular lipids accumulation. PGC-1α is a master regulator of mitochondrial biogenesis [18] and is down-regulated in response to adverse metabolic environments, such as HFD-consumption, due to fatty acid accumulation [10]. Maternal obesity or post-weaning over-nutrition did not impact on *Pgc1α* levels, consistent with preserved oxidative capacity. Myogenic factors, critical for myocyte differentiation and development, are down-regulated in offspring from obese sheep [35]. Here, both *Myog* and *myoD* levels were altered, mainly by maternal obesity, suggesting an initial step towards insulin resistance as decreased *Myog* and *myoD* have been associated with impaired insulin-signalling [13]. Myogenesis occurs only in late gestation and during the neonatal period in rodents [35] and ceases during adulthood, potentially explaining the absence of impact of post-weaning over-nutrition on those myogenic markers. In the present experiment, the down-regulation of the myogenic factors *myoD* and myogenin was associated with an increased body and muscle mass in offspring from obese dams, in line with our previous reports [8]. Both myogenin and *myoD* are involved in muscle hyperplasia. However, decreased levels of myogenin has been reported in weaning rats without any significant impact on muscle mass [2], suggesting that other contributing factors can increase muscle mass. The accumulation of
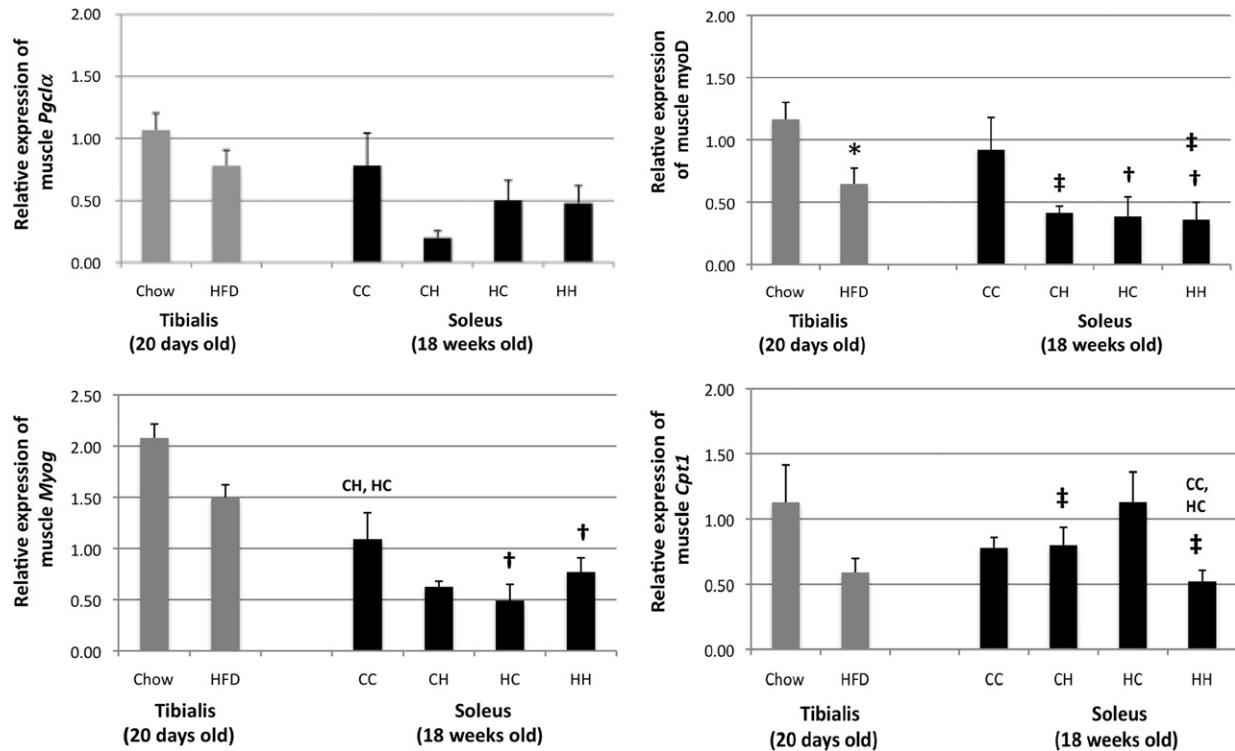
Figure 3 Expression of myogenic markers and lipid transporters. $Pgc1\alpha$: Peroxisome proliferator-activated receptor gamma coactivator-1 alpha, $myoD$: myogenic differentiation protein, $Myog$: myogenin, $Cpt1$: carnitine palmitoyl transferase-1, HFD: high fat diet, CC: mother on chow diet and post-weaning chow diet, CH: mother on chow diet and post-weaning HFD, HC: mother on HFD and post-weaning chow diet, HH: mother on HFD and post-weaning HFD; ∗ significant difference with Chow, $p < 0.05$; † maternal effect, $p < 0.05$; ‡ post-weaning diet effect, $p < 0.05$; CC significant difference with CC, $p < 0.05$; CH significant difference with CH, $p < 0.05$; HC significant difference with HC, $p < 0.05$.

intramuscular fat could partially contribute to the increased muscle mass observed in offspring from obese dams. It must also be noted that the overload generated by increased body weight might stimulate muscle hypertrophy, previously reported to be independent of $myoD$ or myogenin [25].

Although, oxidative capacity was preserved in response to both maternal obesity and post-weaning over-nutrition, GLUT4 levels were decreased suggesting a defect in fuel metabolism. During lipid oxidation, transfer of fatty-acyl CoA to the mitochondria through CPT-1 represents the rate-limiting step [16]. The down-regulation of $Cpt1$ in response to both maternal obesity and post-weaning over-nutrition suggests a defect in this process, potentially causing lipid metabolites accumulation, leading to insulin-signalling defect and reduced GLUT4 in skeletal muscle [4,31]. One limitation of the study is that the physical activity levels of our rats and AMP-activated protein kinase (AMPK) activity, controlled by muscle contraction, were not measured. As AMPK regulates lipid oxidation by controlling CPT-1 and mitochondrial biogenesis [4], it cannot be ruled out that a reduced AMPK activity could contribute to our results. However, a recent report showed that reduced AMPK activity did not exacerbate the development of insulin resistance in HFD-induced obesity [4], supporting the need for further investigations.

Hyperlactatemia has been reported in obesity and insulin resistance and represents an independent predictor of insulin resistance [28]. Skeletal muscle is the main producer and consumer of lactate, with glycolytic fibres generating and releasing most of the lactate through MCT4, while oxidative fibres taking it up through MCT1 for oxidation. A defect in lactate transport could contribute to hyperlactatemia and then to insulin resistance [23]. Weaning rats showed increased MCT1 levels, whereas over-nourished adult rats had decreased levels. This was associated with increased LDH activity, suggesting increased glycolysis and thus increased lactate production. Insulin stimulates glucose uptake and glycolysis, and not only skeletal muscle but also adipose tissue can produce lactate providing two mechanisms by which obesity leads to lactate over-production [28]. Lactate can be metabolised mainly in the heart, liver and skeletal muscles, and specifically in oxidative fibres where MCT1 is mostly expressed. Thus, the increase in MCT1 in weaning rats from obese mothers could represent an attempt to increase blood lactate clearance. However, reduced MCT1 in adult rats in the face of increased glycolysis could underpin the hyperlactatemia observed in HFD-fed rats, contributing to exacerbated insulin resistance in skeletal muscles. In our study, glycolytic muscles seemed to be better protected against metabolic disturbances compared to oxidative muscles in line with previous reports suggesting a fibre type specific susceptibility to insulin resistance [6,7,15]. Although the exact mechanisms are still unknown, an increased fatty acid transport capacity has been reported in oxidative versus glycolytic muscles and was inversely correlated with

GLUT4 levels in the same muscles, suggesting a major role in the development of insulin resistance [6].

In summary, we show that a defect in glucose transporters and impaired myogenesis resulting in systemic insulin resistance is directly linked to both maternal obesity and post-weaning over-nutrition. Although some beneficial adaptations are observed at weaning in response to maternal obesity, mainly through increased muscle oxidative capacity, they fail to prevent muscle metabolic dysfunction. The imposition of post-weaning over-nutrition amplifies metabolic dysfunction and overrides the adaptations observed at weaning. This suggests the nutritional environment during adulthood remains the main risk factor for the development of muscle metabolic dysfunction, while "metabolic programming" by maternal obesity can predispose individuals to the development of insulin resistance and diabetes.

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