AOGS
ACTA Obstetricia et Gynecologica Scandinavica

AOGS MAIN RESEARCH ARTICLE

Acute and perinatal programming effects of a fat-rich diet on rat muscle mitochondrial function and hepatic lipid accumulation

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Key words
High-fat diet, maternal diet, respiratory coupling ratio, non-alcoholic fatty liver, metabolic syndrome

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Conflict of interest
The authors have stated explicitly that there are no conflicts of interest in connection with this article

Please cite this article as: Hellgren LI, Jensen RI, Waterstradt MSG, Quistorff B, Lauritzen L. Acute and perinatal programming effects of a fat-rich diet on rat muscle mitochondrial function and hepatic lipid accumulation. Acta Obstet Gynecol Scand 2014; 93: 1170–1180.

Received: 7 July 2014
Accepted: 17 July 2014

DOI: 10.1111/aogs.12458

Abstract

Objective. Maternal high-fat intake during pregnancy may have long-term consequences in the offspring. Since this might relate to the capacity of mitochondrial metabolic adaptation and hepatic lipid metabolism, we investigated how maternal high-fat intake affected mitochondrial function and hepatic steatosis in the offspring. *Design.* Sprague–Dawley rats were fed a high-fat (20% w/w) or a control diet (chow, C) from 10 days before pregnancy and throughout lactation. At weaning the litters were split into two groups; one was continued on the maternal diet and the other was fed low-fat chow. *Sample.* Skeletal muscle mitochondria and liver lipids. *Methods.* Mitochondrial respiration and hepatic lipid content were determined during and after weaning, on days 20 and 70 postpartum. *Main outcome measures.* Mitochondrial function and hepatic lipids. *Results.* At 20 days, maternal high-fat diet caused increased $V_{O_{2max}}$ with pyruvate as substrate ($p = 0.047$), at 70 days, pups born by C-dams, but not those born by high-fat-dams, showed increased oxidation of palmitoylcarnitine in the absence of ADP ($p = 0.018$). Rates of ADP-stimulated oxygen consumption, maximal respiratory capacity and mitochondrial respiratory control ratio with pyruvate, increased post weaning ($p < 0.001$), whereas respiratory control ratio with palmitoylcarnitine decreased ($p = 0.013$). The increase in respiratory control ratio was most pronounced in pups from C-dams ($p = 0.05$). The high-fat-diet caused pronounced hepatic steatosis in pups at weaning ($p < 0.001$), without concomitant ceramide accumulation, while high-fat-feeding after weaning induced triacylglycerol and ceramide accumulation ($p < 0.01$), regardless of maternal diet. *Conclusion.* Intake of a fat-rich diet during pregnancy and lactation reduced the age-induced increases in un-coupled fat oxidation.

Abbreviations: C, control; CS, citrate synthase; HF, high-fat; PC, palmitoylcarnitine; RCR, respiratory control ratio; TAG, triacylglycerol; $V_{O_{2max}}$, maximal respiratory capacity.

Introduction

In recent years, there has been an increased focus on the link between maternal fat intake during pregnancy and metabolic dysfunctions, such as obesity and insulin resistance, in the later life of the offspring (1). In animal-models, maternal obesity and/or intake of a fat-rich diet during pregnancy can program adiposity (2), insulin resistance and hepatic steatosis (3,4), as well as hypertension

(2) and dyslipidemia in the offspring (5). In human studies, maternal pre-pregnancy obesity has been associated to increased obesity and cardiovascular dysfunction, as well as insulin resistance and hepatic steatosis, in the childhood, adolescence and adult life of the child (6–10).

Although the molecular mechanisms leading to this metabolic programming still are unclear, mitochondrial dysfunction seems to be involved. It has been shown that adult insulin resistance in the offspring of mothers fed a fat-rich diet is preceded by reductions in mitochondria number and mitochondrial genome expression (11). Furthermore, both mitochondrial electron transport chain activity and expression of genes coding for proteins in the electron transport chain complex are reduced in the offspring (12). In a sheep model, fat-rich diets postpartum induced an increase in maximal respiratory capacity ($V_{O_{2max}}$) and mitochondrial respiratory control ratio in the lamb, indicating effects on mitochondrial function (13). Reduced mitochondrial activity reduces the capacity of mitochondrial fatty acid oxidation, which can lead to accumulation of lipid intermediates, such as ceramide, that directly or indirectly attenuate signal transduction from the insulin receptor in the liver and skeletal muscles (14,15). Thus, the observed reduced mitochondrial activity in the offspring from obese-mothers can potentially mechanistically explain their disadvantageous metabolic imprinting. Hepatic steatosis is strongly correlated to whole-body insulin sensitivity both in obese and non-obese individuals, and epidemiological data suggest that a fatty liver predicts development of metabolic syndrome, independent of other risk markers (16). The etiology of hepatic steatosis is not fully understood but it reflects an imbalance between the input of fatty acids to the liver, through uptake from the circulation and/or de novo synthesis, and hepatic fatty acid output in the form of oxidation and very low density lipoprotein secretion. It has been shown that fatty acid uptake from the circulation is the main source of liver fat in humans suffering from non-alcoholic fatty liver disease, although increased de novo lipogenesis and reduced oxidative capacity also have substantial impact (17).

Although hepatic steatosis is normally assessed through quantification of triacylglycerol (TAG), other lipid-intermediates, formed during increased fatty acid load in the liver, are considered to be the true metabolic culprits (18). Ceramide is one such lipid-intermediate that has gained considerable interest due to its association with type-2 diabetes in humans (19). Ceramide is an intermediate in the metabolism of sphingolipids that attenuates signaling from the insulin receptor at the level of protein kinase B/Akt (20), as well as inducing oxidative stress and promoting tissue inflammation (19,21). Ceramide levels in the liver are normally considered to be closely associated to fatty acid load (21) and plasma ceramide is strongly associated to insulin resistance in humans (19).

It is known that an obesogenic fat-rich diet during pregnancy and lactation has long-lasting effects on hepatic fat in the offspring in both rodents and non-human primates (4,22). We recently showed that maternal intake of an obesogenic diet causes increased TAG and ceramide accumulation in the liver of mice fetuses (23), but very little is known about how maternal fat intake can affect hepatic ceramide content in the offspring after birth.

In the present study, we tested the hypothesis that maternal intake of a fat-rich diet during pregnancy and lactation alters mitochondrial function, which will be associated to increased tissue accumulation of ceramide and insulin resistance in early adult life. We have therefore studied the function of skeletal muscle mitochondria and hepatic TAG and ceramide concentrations in offspring at an early stage.

Material and methods

Animal husbandry and experimental diets

All animal studies were approved by the Danish Animal Experiments Inspectorate and were carried out according to the guidelines of the Council of Europe Convention for the Protection of Vertebrate Animals used for Experimental and other scientific purposes. Female Sprague–Dawley rats (100 days of age) were maintained in 12 h:12 h light-dark cycles at constant temperature and humidity and fed ad libidum either a control (C) or an experimental high-fat (HF) diet from 10 days before pregnancy, throughout pregnancy and during lactation (Figure 1). At 48 h postpartum, each of the litters produced (11 for C and 10 for HF) was reduced to eight pups per litter, of which at least five were males. At 20 days of age, mitochondrial function was studied in one male offspring from each litter. Although gender differences in the outcomes are possible and would be interesting to study, economical and physical constraints in the animal facility did not allow us to do this in present study therefore female offspring were sacrificed at

Key Message

Skeletal muscle mitochondrial function in offspring from mother animals fed a high-fat diet during gestation and lactation, do not support the pre-adaptive response hypothesis, since these pups did not have increased capacity ADP-stimulated fatty acid oxidation but had a reduced uncoupled fatty acid oxidation.

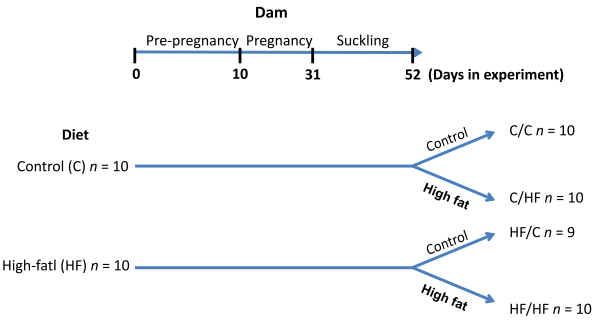

Figure 1. Outline of the feeding study. Dams were fed either a high-fat or control diet from 10 days before pregnancy, throughout pregnancy and during lactation. After weaning, the male offspring from each diet group were either continued on the maternal diet or switched to the opposite diet.

weaning (21 days postpartum). After weaning, two male offspring from dams fed the control diet were continued on the control diet (C→C), while another two were transferred to the high-fat diet (C→HF). Four male offspring from the dams fed the fat-rich diet were split in a similar fashion into one group fed the control diet after weaning (HF→C) and one group fed the high-fat diet (HF→HF). The resulting four experimental groups had $n = 10$ as shown in Figure 1.

The control diet consisted of a standard laboratory chow and mixture of vitamins and minerals (Rat and Mouse Diet no. 3, Special Diet Services, Witham, Essex, UK). The experimental high-fat diet consisted of the standard chow supplemented with 20% (by weight) palm oil and correspondingly increased amounts of vitamins and minerals, protein, inositol, and choline. Palm oil was chosen, since we wanted to study the effect of a largely saturated fat that is common in the human diet. As shown in Table 1, this meant that the HF-diet had a higher energy density, a slightly reduced relative protein and carbohydrate content, and an increased n-6/n-3 ratio. Both diets contained equal amounts of vitamin E (193 mg/kg). Food intake and body weight were recorded throughout pregnancy and weaning, and up to 70 days of age in the offspring. The plasma insulin level in the offspring was measured in plasma from un-fasted animals at 70 days of age using a rat insulin ELISA (Mercodia AB, Uppsala, Sweden).

Table 1. Energy and fat content in the HF and C diet.

Fatty acids	HF	C
kcal/g	20.1	15.1
Carbohydrate (%w/w)	34.7	38.3
Protein (%w/w)	19.5	22.5
Total fat (%w/w)	22.7	4.9
SFA	10.2	0.9
MUFA	8.5	1.0
PUFA	3.8	2.8
n-6/n-3 PUFA	10.3	5.7

HF, high-fat; C, control; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acid.

Preparation of isolated mitochondria from skeletal muscle

One male offspring from each litter and each dietary group was sacrificed by cervical dislocation at day 20 and day 70, – ideally $n = 10$ per group. However, some of the mitochondrial isolations and analyses of some of the mitochondrial parameters were not successful (the exact number of rats for each parameter is given in Results). Mitochondria were prepared as described earlier (24); in brief, the quadriceps muscle from both hind legs was removed and homogenized using a Potter Elvehjem homogenizer (Cole-Parmer, Vernon Hills, IL, USA). Following centrifugations, the final mitochondrial pellet was resuspended in 1 mL of MS-medium (mannitol 225 mM, sucrose 75 mM) and used for the measurements of O_2 consumption as well as for analysis of citrate synthase (CS) activity (25) and protein concentration (26).

Mitochondrial respiration

Respiration was determined in triplicate in high-resolution respirometers (Oroboros Oxygraph, Innsbruck, Austria) at 25°C (27). The analysis buffer contained 2.0 mL $MSTP_i$ buffer (mannitol 225 mM; sucrose 75 mM; Tris-base 20 mM; EDTA 0.5 mM and KH_2PO_4 10 mM; pH 7.0) and was gassed with 95% oxygen and 5% CO_2. Two protocols were used: (i) malate 2 mM; pyruvate 0.5 mM; ADP 3 mM; succinate 5 mM; and (ii) malate 2 mM; palmitylcarnitine (PC) 10 µM; ADP 3 mM; succinate 5 mM, ensuring steady state respiration between substrate additions (28). State 3 and State 4 respiration are defined as the oxygen consumption with and without the presence of ADP, respectively. Thus, State 4 respiration represents electron transport not coupled to ATP synthesis. The maximal respiratory capacity, $V_{O_{2max}}$, was taken as the State 3 respiration with further addition of 5 mM succinate, ensuring substrate saturation of both complexes I and II of the respiratory chain. Oxygen consumption was expressed relative to both mitochondrial protein content and CS activity, as proxy for the mitochondrial mass (29). However, since CS activity per mg mitochondrial protein was similar in the groups (Table 2), data are only shown normalized to mitochondrial protein. Respiratory control ratio was calculated as the ratio between State 3 and State 4 respiration.

Table 2. Mitochondrial citrate synthase activity and protein content in the different dietary groups.[a]

	Day 20				Day 70		
	CS activity (U/mL)	Protein (mg/mL)	CS/protein (U/mg)		CS activity (U/mL)	Protein (mg/mL)	CS/protein (U/mg)
C	12.8 ± 1.4 (8)	8.71 ± 1.07 (8)	1.49 ± 0.26 (7)	C→C	10.4 ± 1.6 (11)	6.95 ± 1.08 (11)	1.52 ± 0.30 (11)
				C→HF	10.6 ± 1.9 (10)	7.59 ± 1.63 (10)	1.49 ± 0.55 (10)
HF	13.6 ± 3.0 (8)	8.28 ± 1.23 (9)	1.69 ± 0.45 (8)	HF→C	10.9 ± 2.0 (10)	7.00 ± 2.03 (10)	1.66 ± 0.50 (10)

CS, citrate synthase; C, control; HF, high-fat.
[a]Data are given as mean ± SD (*n*).

Liver TAG and ceramide

Lipid extraction, isolation of TAG, phospholipid free fatty acids, formation of fatty acid methyl-esters and subsequent analysis by gas-chromatography as well as ceramide analysis, were performed as described by Hou et al. (30). Glyceryl trinonadecanoate, nonadecanoic acid, glycerophosphorylcholine di-nonadecanoyl (all from Sigma, Brøndby, Denmark) and a ceramide with a C-17 long-chain base (Avanti Polar Lipids, Alabaster, AL, USA) were added before extraction and used as internal standard in the quantitative analysis. For the ceramide analysis, an external standard curve was applied to ensure linear detector response in the relevant range.

Statistical analysis

The data were normally distributed and the differences in mitochondrial function as well as the liver TAG and ceramide between the dietary groups at days 20 and 70 were therefore analysed using an ANCOVA, analyzing the effect of maternal and post-weaning diet separately and also including a maternal × post-weaning diet interaction term. The values from day 20 were used as covariates and those from day 70 as the dependent variable. For calculations of delta-values in Table 3, animals from day 70 were paired with siblings at day 20. Due to the missing values in some animal pairs and of some of the mitochondrial parameters, statistical calculations of mitochondrial function at day 70 were also performed with mother-ID as a covariate instead of day 20 values in siblings (thus taking the paired design into account). Results from both analyses are given in Table 3.

Results

Maternal and offspring weight gain and food intake

No significant difference was observed between the groups in terms of duration of pregnancy and litter size (data not shown). The fat-rich diet did not cause any changes in maternal food intake or weight gain relative to controls (Figure 2). Thus, at pregnancy day 20, dams from both groups weighed on average 421 g and the total energy intake was 11 420 ± 1940 and 10 040 ± 2400 kJ in control and HF-dams respectively. Similarly, weight gain and food intake in the pups from 21 to 70 days of age were the same in all four groups (Figure 3). Maternal diets did not affect plasma insulin concentrations, but post-weaning HF-diet reduced non-fasting plasma insulin at 70 days of age, regardless of maternal diets ($p = 0.02$) (C→C 1.36 ± 0.21 (7) μg/L, C→HF 0.96 ± 0.17 (10) μg/L, HF→C 1.80 ± 0.40 (9) μg/L, HF→HF 0.88 ± 0.11 (7) μg/L [mean ± SE (*n*)].

Mitochondrial respiratory coupling and respiratory capacity

To reveal how the dietary treatments affect mitochondrial oxidation of carbohydrate- and fatty acid-derived substrates, the capacity to oxidize both pyruvate and palmitoylcarnithine was assayed. Generally, all mitochondrial parameters were higher in the offspring of the maternal high-fat group at 20 days of age, but the only significant difference relative to the control group was in the maximal respiratory capacity [defined as $V_{O_{2max}}$ in the presence of ADP (State 3 respiration) and succinate] with pyruvate as substrate (Table 3). Oxygen consumption under this condition was 82 ± 8 nmol compared with 110 ± 10 nmol O_2/(min*mg protein) in the control group and the HF group, respectviely. Furthermore, across both groups the respiratory control ratio and $V_{O_{2max}}$ with PC (to assay fatty acid oxidation capacity) was significantly lower than that with pyruvate at both 20 and 70 days of age. This difference increased with age, as respiratory control ratio with pyruvate as substrate increased from ~5 at 20 days to ~12 at 70 days of age. Oxidation of the fatty acid substrate (PC) was, on the other hand, slightly decreased, going from a value around 4 at 20 days to an average of 2.6 at 70 days. There was also an age-dependent increase in the ADP-stimulated (State 3) respiration of pyruvate, whereas the ADP-stimulated oxidation of PC tended to decrease and the respiration in the absence of

Table 3. Oxygen consumption of isolated mitochondria from the quadriceps muscle of rats fed either a control low-fat diet (C) or a high-fat diet (HF) during pregnancy and after 20 days *post-partum.*[ab]

	Day 20			Day 70						
	C	HF	p C vs. HF	C→C	C→HF	HF→C	HF→HF	p-value Maternal diet effects[c]	p-value Post-weaning diet effects	p d20 vs. d70
State 4										
With Pyruvate	11.9 ± 0.7 (7)	12.4 ± 0.9 (8)	NS	11.2 ± 0.9 (10)	11.1 ± 1.6 (11)	16.6 ± 4.0 (10)	15.8 ± 3.2 (10)	NS	NS	NS
ΔState 4				−1.4 ± 0.6 (7)	−0.7 ± 1.0 (7)	−0.2 ± 1.0 (8)	1.1 ± 2.5 (8)	NS	NS	
With PC	10.2 ± 1.4 (7)	14.9 ± 2.9 (8)	NS	15.2 ± 1.5 (10)	16.4 ± 2.3 (10)	14.6 ± 1.8 (10)	13.1 ± 1.5 (10)	NS	NS	NS
ΔState 4				5.9 ± 1.6 (7)	8.6 ± 2.3 (7)	−2.1 ± 3.6 (8)	−1.7 ± 2.5 (8)	***0.018***[d]	NS	
State 3										
With Pyruvate	65 ± 0.5 (7)	87 ± 9 (8)	0.082	135 ± 10 (10)	125 ± 15 (11)	136 ± 10 (10)	122 ± 15 (10)	NS	NS	***<0.001***
ΔState 3				67 ± 7 (7)	66 ± 7 (7)	50 ± 11 (8)	52 ± 7 (8)	0.096	NS	
With PC	42 ± 8 (7)	69 ± 13 (8)	NS	34 ± 3 (10)	45 ± 9 (10)	33 ± 7 (10)	39 ± 8 (10)	NS	0.052	0.055
ΔState 3				−9 ± 9 (7)	12 ± 13 (7)	−34 ± 19 (8)	−29 ± 18 (8)	NS	NS	
Vo_{2max}										
With Pyruvate	82 ± 8 (7)	111 ± 10 (8)	***0.047***	175 ± 12 (10)	160 ± 19 (11)	182 ± 14 (10)	159 ± 20 (10)	NS	NS	***<0.001***
ΔVo_{2max}				89 ± 10 (7)	88 ± 9 (7)	71 ± 13 (8)	69 ± 8 (8)	0.054	NS	
With PC	58 ± 9 (7)	94 ± 16 (8)	0.093	51 ± 5 (10)	66 ± 13 (10)	51 ± 10 (10)	60 ± 13 (10)	NS	0.087	NS
ΔVo_{2max}				−8 ± 11 (7)	20 ± 17 (7)	−38 ± 26 (8)	−33 ± 23 (8)	NS	NS	
RCR										
With Pyruvate	5.3 ± 0.6 (8)	6.9 ± 1.4 (9)	NS	13.4 ± 0.9 (11)	12.7 ± 1.1 (11)	11.1 ± 1.4 (10)	10.9 ± 1.6 (10)	NS	NS	***<0.001***
ΔRCR				8.0 ± 0.8 (8)	6.8 ± 0.9 (8)	5.2 ± 0.8 (9)	4.9 ± 1.2 (9)	***0.050***	NS	
With PC	3.6 ± 0.4 (8)	4.5 ± 0.9 (9)	NS	2.5 ± 0.3 (11)	2.8 ± 0.4 (11)	2.4 ± 0.5 (10)	2.8 ± 0.5 (10)	NS	NS	***0.013***
ΔRCR				−1.2 ± 0.7 (8)	−0.3 ± 0.6 (8)	−2.0 ± 1.2 (9)	−1.4 ± 1.1 (9)	NS	NS	

[a]Mitochondrial respiration is given as nmol O_2/(min*mg protein). State 3 is defined as the oxygen consumption with substrate plus ADP, while state 4 respiration is without ADP. RCR is defined as the state 3/state 4 ratio. $Vo_{2\ max}$ is defined as state 3 respiration in the presence of 5 mM succinate ensuring contribution of complex I as well as complex II to the oxygen consumption.

[b]Data are given as mean ± SE (n = 8–10).

[c]All groups consist of one offspring per dam sacrificed at 20 or 70 days of age. p-values for day 70 are given both for analyses where mother-ID was used to consider the paired design (upper line) and where this was done by inclusion of day 20-values for the sibling, i.e. the Δ-values (in lower line).

[d]Values given in ***bold italics*** signifies p-values <0.05.

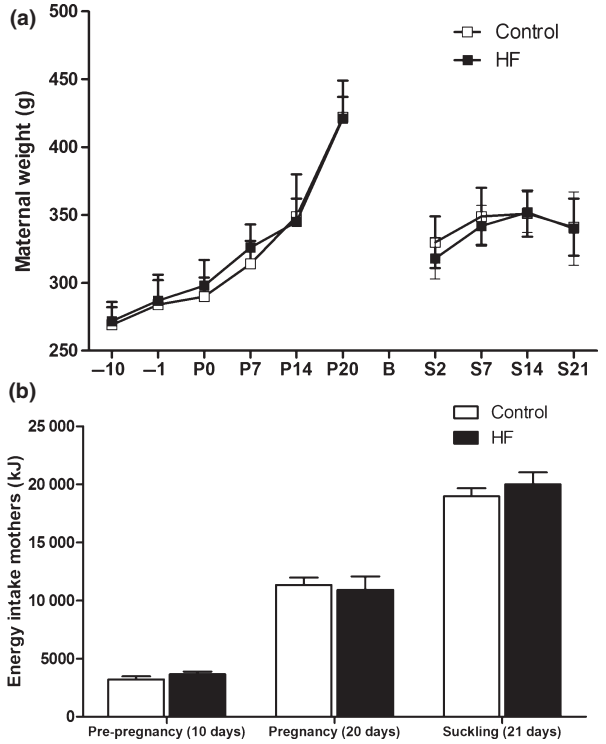

Figure 2. Food intake and weight gain in the dams. Weight (a) and food intake (b) during pregnancy and lactation of dams fed either a control ( ) or a high-fat diet (■) from 10 days before mating (−0 to −1), throughout pregnancy (P0–P21) and during lactation (S1–S21). The results are shown as mean ± SD, n = 10 in both groups.

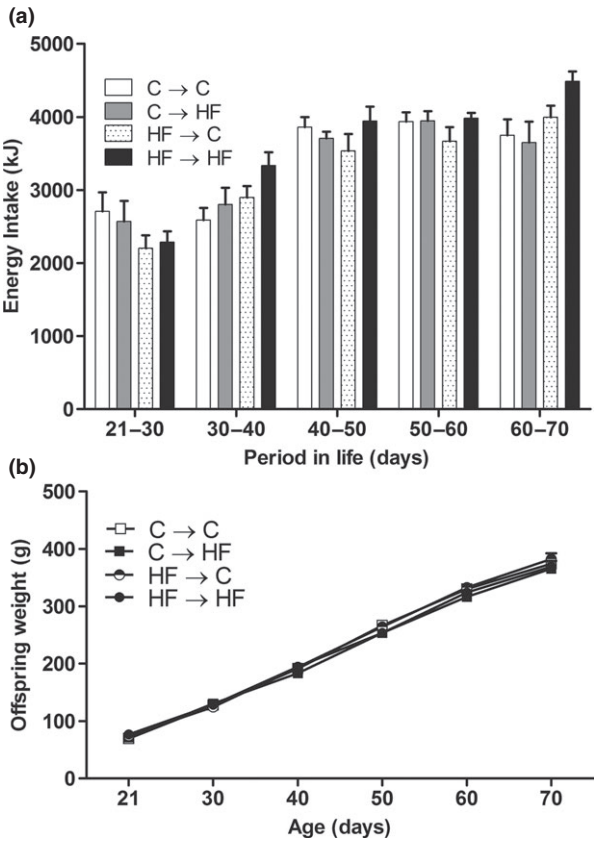

Figure 3. Food intake and weight gain in offspring. Weight gain (a) and food intake (b) from 20 to 70 days of age in male offspring of mothers who during the maternal period were fed a high-fat (HF) or a control diet (C) and who themselves were given the control (C→C and HF→C) or the fat-rich diet from weaning at day 21 (C→HF and HF→HF). The results are shown as mean ± SD, n = 7–10 in each group.

ADP (State 4) did not show any overall change with age. Finally, $V_{O_{2max}}$ with pyruvate increased from 20 to 70 days of age, whereas no significant change was apparent in maximal oxygen consumption with PC as a substrate.

Analysis of the dietary effects on the mitochondrial parameters at day 70 was done both by maternal pairing and based on the changes from day 20 to day 70 (using sibling values from day 20). Maternal diet had a significant effect on the age-dependent change in the respiration of PC in the absence of ADP (State 4 respiration), as it increased with age from 10.2 ± 1.4 to 15.8 ± 1.9 nmol O_2/(min*mg protein) in the offspring of control dams and slightly decreased, and from 15.8 ± 1.9 to on average 13.9 ± 1.7 in pups by HF-fed dams ($p < 0.05$ for maternal diet effect). The analyses (Table 3) also showed a borderline significant effect ($p = 0.05$) of the maternal diet on the efficacy of the coupling between ATP synthesis and mitochondrial electron transport in the oxidation of carbohydrates. The respiratory control ratio with pyruvate as substrate increased less for pups born by HF-dams from day 20 to day 70. Offspring from the maternal C-group increased from 5.3 ± 0.6 to around 13, and the offspring from the HF-group from 6.9 ± 1.4–11. The post-weaning diet tended to result in increased fatty acid-driven (PC as substrate) ADP-stimulated respiration ($p = 0.052$) and $V_{O_{2max}}$ ($p = 0.087$) in pups from the HF-dams at day 70.

Hepatic lipid content

Despite the dams from the two groups having an identical caloric intake during pregnancy and lactation, the pups from the dams fed the fat-rich diet had a significantly higher hepatic TAG content after 20 days (Figure 4). Thus, the average hepatic TAG level in these pups was 43 ± 11 mg/g, whereas it was 12 ± 5.2 mg/g in the pups from dams fed the control diet ($p < 0.0001$). After 70 days, hepatic lipid levels were normalized in the pups that shifted from the fat-rich maternal diet to the control diet after weaning, resulting in a TAG-concentration of 6.6 ± 1.6 mg/g in the HF→C group and 6.2 ± 1.2 mg/g in the C→C group. Shifting from maternal control to fat-rich diet after weaning, on the other

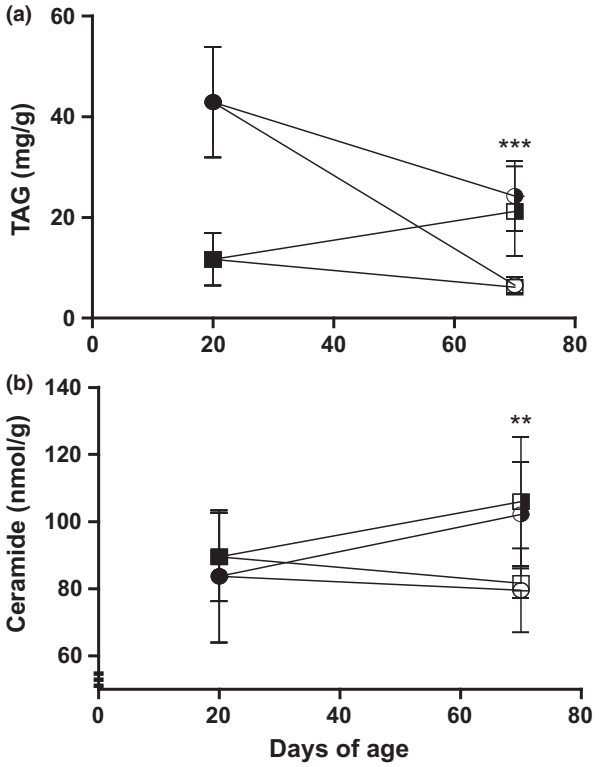

Figure 4. Hepatic lipid content in the offspring. Triacylglycerol (TAG) (a) and ceramide (b) content in the livers from male offspring born to dams fed either control diet (C) (squares) or a high-fat diet (HF) (circles) during pregnancy and lactation. After weaning at day 20 after birth, pups were assigned to receive either the control diet [C→C and HF→C (open symbols)] or the fat-rich diet [C→HF and HF→HF (half-closed symbols)] for 20–70 days of age. Data are given as mean ± SD, n = 7–8, and p-values by asterisks: $^{**}p < 0.01$ and $^{***}p < 0.0001$, respectively, for an effect of the post-weaning diet, analyzed by two-way ANOVA.

hand, led to TAG accumulation similar to the pups raised by the dams fed the fat-rich diet. Average TAG concentration was 21.2 ± 8.9 mg/g in the C→HF group and 24.3 ± 6.9 in the HF→HF group.

At day 20, both groups had identical hepatic ceramide levels of around 90 nmol/g. Intake of the fat-rich diet post-weaning resulted in a ceramide accumulation of about 100 nmol/g after 70 days, regardless of the pre-weaning diets, whereas the two groups given the control diets had levels ~80 nmol/g. The post-weaning diet had a significant effect on both TAG and ceramide content ($p < 0.001$ for TAG TAG and $p < 0.01$ for ceramide).

Discussion

In this study we examined a possible programming effect of a maternal fat-rich diet on skeletal muscle mitochondrial function and hepatic lipid deposition in the offspring at 20 and 70 days of age, corresponding to early childhood and adolescence. The main result of the study was that maternal HF-diet increased the maximal mitochondrial capacity for carbohydrate-derived substrate, measured as pyruvate oxidation, but not for oxidation of fatty acids, analysed as oxidation of palmitoyl-carnithine, at weaning. Between weaning and 70 days of age, maternal HF-diet resulted in a trend ($p = 0.055$) toward a lower increase in the un-coupled respiration of fatty acids than was observed in rats born to and lactated by control-fed dams. Furthermore, the apparent coupling between substrate oxidation and ATP synthesis (coupling ratio) increased after weaning in oxidation of pyruvate, and this increase tended ($p = 0.05$) to be lower in pups from HF-dams. Furthermore, maternal high-fat intake induced increased levels of TAG, but not ceramide, in the liver of the offspring at 20 days of age, whereas a high-fat diet post-weaning resulted in an increase of both at day 70, regardless of maternal diet. Thus, we observed what appears to be a mitochondrial adaptation to the higher energy density of the maternal diet, which was evident from an overall higher respiratory capacity; this was in particular evident with pyruvate as substrate at weaning.

There was no significant programming effect of maternal diet on mitochondrial function at maturity (i.e. no significant differences at day 70 or interactions between pre- and post-weaning diet). The hypothesis about predictive adaptive response suggests that metabolic capacity adapts to the dietary exposure encountered during fetal or early post-natal life (31). From this, we would expect that offspring that were exposed to high-fat diets during perinatal life to develop a better capacity for fat oxidation compared with offspring from dams fed a low-fat diet. However, in present study the mitochondrial capacity for ADP-dependent fatty acid oxidation did not differ between the groups, although ADP-independent fatty acid oxidation and the coupling ratio for pyruvate increased more in the offspring of dams on the control diet than on the HF-diet. Hence, at early maturity the HF-offspring tended to have mitochondria that were less suited to generate energy from carbohydrates and had a relative reduced futile oxidation of fat, compared with the C-offspring. To the best of our knowledge, there exist no previous published data on substrate-selective effects on respiratory efficacy in the offspring, caused by maternal high-fat intake during pregnancy and lactation. It is, however, known that high-fat feeding of young rats for 15 days at weaning improves the capacity for fatty acid oxidation in skeletal muscle mitochondria (32). Together with our data, this indicates that mitochondrial substrate adaption is not aligned with the hypothesis on predictive adaptive response but is rather a direct effect of the macronutrient composition in the diet of the offspring.

The programming effects of maternal high-fat diets during gestation on mitochondrial number, as well as the expression and activity of proteins related to oxidative phosphorylation in the offspring, have been investigated in several studies. Taylor et al. (11) observed a decrease in kidney mitochondrial copy number and in mitochondrial gene expression in the aorta of adult rat offspring from high-fat fed dams. In mice, maternal high-fat feeding, but not post-weaning high-fat intake, was shown to result in a reduced activity of all four mitochondrial electron transport-chain complexes in liver mitochondria from pups at 15 weeks of age (12). Shelley et al. (33) also reported that maternal HF-diet during gestation and lactation caused reduced activity in skeletal muscle mitochondrial complex II–III activity in male offspring but not female offspring. Recently, pathway enrichment analysis in a global transcriptomic study in skeletal muscles from 1-year-old rats born and lactated by HF- or C-fed rats, showed that oxidative phosphorylation is strongly down-regulated at both the mRNA and protein level in rats born by high fat-fed dams (34). In all these studies, as well as in present one, the HF-intervention was done throughout gestation and lactation. Different tissues differentiate and develop in different time-windows during pregnancy, and this also differs between species. It has therefore been suggested that different organs have different "windows of susceptibility" with regard to fetal programming (35). For example, it was recently shown that malnutrition after the primary and secondary myotube formation does not cause metabolic dysfunction in skeletal muscles (30), but did so in the liver (36) in a sheep-model of fetal programming. It is therefore plausible that the outcomes might have been different had the interventions been limited to only a part of the pregnancy or had the HF-pups been cross-fostered by C-dams, and vice versa, after birth.

Increasing the fat amount in the diet inevitably leads to a relative reduction in the protein and carbohydrate content. Protein malnutrition (8 weight% protein in the diet) of rat dams during gestation and lactation has been shown to reduce mitochondrial DNA content and the expression of mitochondrial DNA-encoded genes in the liver and muscle of the offspring up to 20 weeks of age (37). In a sheep model it has also been shown that 50% food restriction during the last trimester of pregnancy reduced ADP-independent respiration, $V_{O_{2max}}$ and respiratory control ratio with pyruvate in the adult offspring (13). Although protein content in the HF-group was slightly lower than in the C-group in present study (19.5 w% vs. 22.5 w%, Table 1), we find it very unlikely that this minor difference could lead to the observed alterations in mitochondrial function.

The original thrifty phenotype hypothesis suggested that the detrimental effects were only seen if the postnatal diet did not match with the conditions during fetal life. Our results on mitochondrial function did not indicate any effect of a change in diet at weaning; however, the results did indicate long-term detrimental effects of an energy-dense maternal diet. It is therefore possible that energy surplus, as well as energy restriction, may reduce metabolic health in the offspring.

Concerning hepatic TAG concentrations, our results are in agreement with a recent study by McCurdy et al. (4), who showed that feeding macaque mothers a high-fat diet during gestation and lactation resulted in hepatic fat accumulation in the offspring. Thus, high maternal fat intake seems to be an important contributor to early onset fatty liver in the offspring. However, in our study, liver fat levels were fully reversed to the level in the control pups in the animals that were transferred from the HF-diet to chow-control at weaning. Thus, a high fat intake during gestation and lactation did not induce any programming effect that was detectable in early adulthood. This is partly in contrast to what has been found in other studies. Bayol et al. (38) have recently shown that male rat pups from dams that have been fed a junk food diet during gestation and lactation followed by a chow-diet post-weaning, had slightly increased liver TAG -levels, compared with the pups from dams that had been given chow through the entire study (38). Since the dietary regimes were different in the two studies, this discrepancy could be explained by a slower rate of reversal to normal lipid levels under the conditions used in the Bayol study than in our study. However, in agreement with the present study, they did not see any effect of the maternal diets on hepatic TAG level in pups that had been fed an energy-dense diet after weaning. This indicates that intake of energy-dense diets after weaning is such a strong modulator of hepatic lipid accumulation that any programming effect induced by maternal diet becomes irrelevant.

A high-fat post-weaning diet was found to result in increased hepatic fat deposition and a decrease in plasma insulin. The difference in insulin was seen in the unfasted state and is therefore most likely an acute effect of the high dietary fat content and thus a lower postprandial insulin response. The molecular mechanism of the high-fat induced insulin-resistance is not known, but several different lipid metabolites have been implicated. In present study, the high-fat diets were based on palm oil, rich in palmitic acid. Palmitic acid stimulates ceramide synthesis, and inhibition of ceramide synthesis has been shown to be sufficient to protect from high palmitate-induced insulin resistance in mice-models (39). Furthermore, sphingolipid metabolism has recently been shown to play a key role in the control of lipid and glucose metabolism in hepatocytes (40). It is therefore

noteworthy that the high maternal fat intake during gestation and lactation caused a pronounced increase in liver TAG-concentration in the offspring at weaning, without affecting the level of ceramide, whereas a lesser degree of high dietary fat-induced liver steatosis after weaning caused a significant increase in ceramide. Thus, our results indicate that the very young liver may be able to cope with higher fat levels without increased formation of detrimental metabolites such as ceramide, whereas this is not the case for the liver of older animals. Very little is known about how hepatic lipid-load affects ceramide levels in the liver of young animals, but it is generally assumed that increased accumulation of lipotoxic lipid-intermediates, including ceramide, occurs when the fatty acid influx is higher than the β-oxidation capacity or the rate of synthesis of membrane and storage lipids. Our results indicate that high hepatic TAG concentration is not linked to lipotoxicity in early life. If this is the case, a dietary shift after lactation from an energy-dense fat-rich diet to a diet containing less fat could protect the offspring from the deleterious effects of fetal and post-natal exposure to high fat levels. However, it was recently shown that fetuses from mice dams fed a high-fat obesogenic diet had increased levels of hepatic ceramide 1 day prior to birth (23). The response in the very young liver might therefore be species-dependent.

The effects on hepatic lipid load and mitochondrial energetics observed with the HF-diet might depend on the fatty acid composition of the fat source. In the present study we used a palm oil-based diet rich in palmitic acid. As already mentioned, palmitic acid is known to stimulate ceramide synthesis, and hence the pathological developments associated to increased ceramide accumulation. The results might have been different if the diet had been rich in polyunsaturated fatty acids. In a recent study, Crescenzo and colleagues (41) showed that HF-diets enriched in n-6 polyunsaturated fatty acids (safflower oil) caused a less pronounced increase in APD-dependent respiration than observed in animals fed an HF-diet enriched in saturated fatty acids (lard). The authors suggest that this is explained by increased proton leakage due to incorporation on arachidonic acid in the mitochondrial membrane phospholipids. Although the experimental design differs substantially from present study, it shows that the acute effects of an HF-diet on mitochondrial function, and hence on whole body energetics, is dependent on dietary fatty acid composition.

The strength of the present study is that it is the first study to determine substrate-specific effects on mitochondrial function in the offspring from high-fat fed mothers. The relatively short follow-up time in the offspring is a significant weakness. It is well known that metabolic dysfunctions increase with age, and it would have been advantageous to have data from older offspring.

In summary, this study found that a maternal palm-oil enriched high-fat diet in utero and during lactation resulted in a pronounced hepatic accumulation of TAG in spite of an increased maximal respiratory capacity in skeletal muscle mitochondria at weaning. High-fat diet intake after weaning led to a lower improvement in respiratory coupling compared with the control diet and tended to increase coupled fat oxidation. In utero and early infancy exposure to a high-fat diet was found to induce a slight reduction in uncoupled respiration of fat. Whether this reduced capacity for uncoupled fat oxidation has any impact on long-time skeletal muscle fatty acid load, and hereby on muscle insulin sensitivity, remains to be determined.

Funding

This research was funded by The Danish Council for Independent Research: Medical Sciences.

Acknowledgments

The TAG and ceramide assessments were conducted in the Department of System Biology, Technical University of Denmark. The mitochondrial experiments were conducted at the Department of Biomedical Science, University of Copenhagen, Denmark. We gratefully acknowledge Pernille Wehler Güllich for help with the animal husbandry and Ib Therkelsen for help with preparation of solutions for the mitochondrial work.

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