Programming effects of an early life diet containing large phospholipid-coated lipid globules are transient under continuous exposure to a high-fat diet

Onne A. H. O. Ronda[1], Bert J. M. van de Heijning[2], Alain de Bruin[1,3], Angelika Jurdzinski[1], Folkert Kuipers[1,4] and Henkjan J. Verkade[1]*

[1]*Department of Pediatrics, University of Groningen, University Medical Center Groningen, 9713 GZ Groningen, The Netherlands*
[2]*Danone Nutricia Research, 3584 CT Utrecht, The Netherlands*
[3]*Dutch Molecular Pathology Center, Faculty of Veterinary Medicine, Utrecht University, 3584 CL Utrecht, The Netherlands*
[4]*Laboratory Medicine, University of Groningen, University Medical Center Groningen, 9713 GZ Groningen, The Netherlands*

Abstract

Breast-feeding is associated with a lower risk of developing obesity during childhood and adulthood compared with feeding infant milk formula (IMF). Previous studies have shown that an experimental IMF (eIMF; comprising Nuturis®) programmed mouse pups for a lower body weight and fat mass gain in adulthood when challenged with a high-fat diet (HFD) compared with a control IMF (cIMF). Nuturis has a lipid composition and structure more similar to breast milk. Here, the long-term effects were tested of a similar eIMF, but with an adapted lipid composition and a cIMF, on body weight, glucose homoeostasis, liver and adipose tissue. Nutrient composition was similar for the eIMF and cIMF; the lipid fractions comprised approximately 50 % milk fat. C57BL/6JOlaHsd mice were fed cIMF or eIMF from postnatal day (PN) 16–42 followed by an HFD until PN168. Feeding eIMF *v.* cIMF in early life resulted in a lower body weight (−9 %) and body fat deposition (−14 %) in adulthood (PN105). The effect appeared transient, as from PN126 onwards, after 12 weeks' HFD, eIMF-fed mice caught up on controls and body and fat weights became comparable between groups. Glucose and energy metabolism were similar between groups. At dissection (PN168), eIMF-fed mice showed larger (+27 %) epididymal fat depots and a lower (−26 %) liver weight without clear morphological aberrations. Our data suggest the size and coating but not the lipid composition of IMF fat globules underlie the programming effect observed. Prolonged exposure to an HFD challenge partly overrules the programming effect of early diet.

Key words: Metabolic programming: Infant nutrition: Dietary lipids: Lipid structure: Milk fat globule membrane: Obesity prevention: Animal models

Breast-feeding is epidemiologically associated with a lower incidence in childhood and adulthood obesity, compared with infant milk formula (IMF) feeding[1]. Breast-feeding is also associated with lower blood pressure and lower plasma cholesterol levels in adulthood[2,3]. The nutritional composition of human milk is mimicked in IMF. However, mimicking fat emulsification is not yet possible. Fat in human milk is dispersed in particles (5 μm diameter) enveloped by a trilayered milk fat globule membrane composed mainly of phospholipids and cholesterol[4]. In contrast, standard IMF fat globules are much smaller (0·1 μm diameter) and coated with surface-adhering proteins[4,5]. Most likely the physicochemical structure (size and coating) of lipid globules in HM serves a biological purpose[4–8]. An experimental IMF (eIMF; Nuturis®) was developed comprising large (mode

diameter 3–5 μm) phospholipid-coated lipid globules similar in size to those in human milk[4,6]. Feeding mice an eIMF-based diet in early life, compared with standard control IMF (cIMF), resulted in a lower body weight and lower fat mass accumulation when fed a high-fat diet (HFD) challenge into adulthood[6,9,10]. The underlying mechanism of this effect on fat deposition of eIMF exposure has not yet been elucidated but is hypothesised to relate to fat globule size and structure.

To confirm and extend previous observations of eIMF programming effects in mouse pups, we used a similar paradigm and determined the possible long-term effects of early life eIMF exposure on body weight accrual, glucose homoeostasis and liver and adipose tissue attributes. We tested the hypothesis that the size and coating of lipid globules, and not the
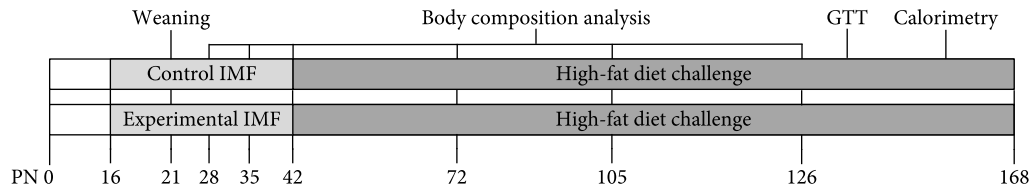
Fig. 1. Study design from postnatal days (PN) 0–168 (*n* 12). GTT, glucose tolerance test; IMF, infant milk formula.

composition, underlie the initial programming effect observed. We therefore compared the cIMF and eIMF with similar lipid compositions.

Materials and methods

Animals and study design

Experimental procedures were approved by an external independent animal experiment committee (Central Animal Experiments Committee, The Netherlands) and complied with the principles of good laboratory animal care following the European Union directive for the protection of animals used for scientific purposes. The present study was conducted in accordance with the institutional guidelines for the care and use of laboratory animals established by the Ethics Committee for Animal Experimentation of the University of Groningen (NVWA 10500) in full compliance with the European Directive 2010/63/EU for the use of animals for scientific purposes. All animals were kept in the same temperature-controlled room (20–22°C, 45–65 % humidity, lights on 8.00–20.00 hours) in type 1L (360 cm²) polysulfone cages bearing stainless steel wire covers (UNO BV), with wood shaving bedding, Enviro-dri® (TecniLab) and cardboard rolls. All mice were handled by the same researcher. Virgin C57BL/ 6JOlaHsd breeders (11 male; 22 female) 12 weeks of age (Envigo) were mated[6] in 2F + 1M couples. Males were removed from couples after 2 d. Pregnancy was confirmed by a >2-g increase in body weight after 1 week and occurred at approximately 66 % efficiency. Delivery day was recorded as postnatal day (PN) 0. Pups were randomised between dams, and litters were culled to 4M + 2F on PN2, weaned at PN21 and diets provided as freshly prepared dough balls (40 % water) from PN16 to PN42[6,9]. Randomisation was not performed as the programming diets were visually distinct. Breeders and female offspring were terminated (using CO₂) at weaning, in compliance with the AVMA Guidelines for the Euthanasia of Animals. From PN42 onwards, male offspring was pair housed with siblings and fed an HFD (45 % energy as fat, D12451; Research Diets Inc.) and tap water *ad libitum* until dissection on PN168. Females were not used, as they are protected against HFD-induced metabolic changes[11]. Glucose tolerance and calorimetry were assessed at PN133 and PN154, respectively (Fig. 1).

Programming diets

Two IMF powders (Nutricia Cuijk B.V.) were tested. The IMF powders had a similar macro- and micronutrient content (Table 1); both lipid moieties comprised about 50 % vegetable

oil and 50 % milk fat (Table 1) and had a similar fatty acid profile (Table 2). cIMF comprised fat globules with a volume mean diameter (D[4,3]) of 0·8 μm, whereas eIMF comprised phospholipid-coated lipid globules with a D[4,3] of 7 μm, explained in more detail elsewhere[4]. IMF powders (283 g/kg feed) were supplemented with protein and carbohydrate (Ssniff Spezialdiäten GmbH) to obtain American Institute of Nutrition (AIN)-93G-compliant diets, with a fat moiety derived entirely from IMF[12].

Body composition

Lean and fat mass were quantified by time-domain NMR (LF90II; Bruker Optics), not requiring fasting or anaesthesia.

Glucose, insulin and pyruvate tolerance tests

Mice were fasted 6, 6 and 4 h for the glucose, pyruvate and insulin tolerance tests, respectively. The glucose tolerance test (intraperitoneal (ip) 13·9 μmol glucose/g body weight), insulin tolerance test (ip 0·5 mU insulin/g body weight) and pyruvate tolerance test (ip 28·4 μmol pyruvate/g body weight) were performed as previously described[13].

Calorimetry

Mice were single housed in a Comprehensive Laboratory Animal Monitoring System (Phenomaster; TSE Systems GmbH) at PN154 for 4 d as previously described[14].

Termination

Mice were anaesthetised (isoflurane/O₂) after a 4-h fasting period (during light phase) and killed by heart puncture; a terminal blood sample was drawn. Liver, epididymal, inguinal, perirenal and interscapular fat were obtained and weighed.

Assays

Plasma was analysed using the V-PLEX Proinflammatory Panel 1 (mouse) kit (K15048D), Mouse Adiponectin kit (K152BXC), Mouse Leptin kit (K152BYC), Mouse MCP-1 Ultra-Sensitive kit (K152AYC), Mouse/Rat Total Active GLP-1, Insulin, Glucagon kit (K15171C) and the Mouse/Rat Resistin kit (K152FNC). Analyses were performed according to the manufacturer's instructions. Kits were purchased from Meso Scale Diagnostics LLC. Blood glucose was measured using a OneTouch Select Plus (Lifescan Inc.).

Table 1. Nutrient composition of the programming diets (postnatal days 16–42) and the high-fat diet (postnatal days 42–168)
(Mean values and standard deviations)

	Control IMF		Experimental IMF		HFD
Carbohydrate*	609		618		396
Mono-/di-saccharides	225		235		172·8
Glucose	3·7		3·4		–
Lactose	134		144		–
Sucrose	85		85		172·8
Other sugars	2·6		2·4		–
Polysaccharides*	380		380		172·8
Maltodextrin	101		101		100
Maize starch	280		280		72·8
Other	0·84		0·68		–
Fibre*	49·0		48·2		50
Cellulose	32·0		32·0		50
Fructo-oligosaccharides	1·7		1·4		–
Galacto-oligosaccharides	15·3		14·3		–
Lipids*	77·2		70·6		203
Vegetable fat	37·5		32·9		25
Milk fat	38·6		36·7		–
Other animal fat	1·1		0·98		–
Lard	–		–		177·5
Phospholipids	0·084		1·1		–
Cholesterol	0·12		0·12		0·20
Protein*	199		198		200
Whey	17·6		16·5		–
Casein	181		181		200
Particle size	Mean	SD	Mean	SD	
D[4,3] (μm)	0·81	0·2	6·8	0·2	–
D[3,2] (μm)	0·43	0·004	0·86	0·1	–
Surface area (m²/g)	15	0·2	7·7	1·0	–

IMF, infant milk formula; HFD, high-fat diet.
* All in g/kg.

Table 2. Fatty acid (FA) composition of the programming diets (postnatal days 16–42)

	Control IMF	Experimental IMF
Saturated*	44	42
14 : 0	8·9	7·1
16 : 0	26	25
18 : 0	7·8	8·9
20 : 0	0·28	0·32
22 : 0	0·27	0·39
24 : 0	0·17	0·26
26 : 0	0·032	0·039
Monounsaturated*	36	39
16 : 1n-7	1·2	1·1
18 : 1n-7	1·9	1·9
18 : 1n-9	33	35
20 : 1n-9	0·38	0·42
22 : 1n-9	0·080	0·13
24 : 1n-9	0·053	0·074
Polyunsaturated*	20	19
n-3 Species	3·4	3·4
18 : 3n-3	2·8	2·8
20 : 5n-3	0·12	0·12
22 : 6n-3	0·38	0·38
22 : 5n-3	0·090	0·099
n-6 Species	16	16
18 : 2n-6	16	15
18 : 3n-6	0·050	Trace
20 : 4n-6	0·44	0·43
20 : 3n-6	0·091	0·12
20 : 2n-6	0·046	Trace
Σn-6 : Σn-3 Ratio	4·8	4·7
20 : 3n-9	0·38	0·42

IMF, infant milk formula.
* All in FA weight%.

Liver fatty acyl chain profiling

Cryogenically crushed liver was homogenised in Potter-Elvehjem tubes. Lipids were *trans*-methylated, extracted and analysed by GC as previously described[15].

Analysis of gene expression

Gene expression was analysed by quantitative real-time PCR as previously described[16]. *Cyclophilin* and *36b4* were used as housekeeping genes for hepatic and adipose tissue gene expression, respectively. Primer and TaqMan probe sequences are given in online Supplementary Table S1.

Histological analysis

A liver lobe and the left epididymal fat pad were formalin fixed and paraffin embedded, sectioned and haematoxylin and eosin stained. Liver slices were scored blindly for (non-alcoholic) steatosis[17] and ballooning[18], and the findings were reviewed by a certified veterinary pathologist (AdB). Liver sections were stained for the proliferation marker Ki-67 as previously described[19]. Histological scoring of Ki-67 was performed in five separate 40× fields by a single assessor. Binucleation and karyomegaly were assessed as described[20]. Adipose tissue sections were quantified using Adiposoft[21]. Adipose tissue

was assessed for the presence of inflammatory foci (crown-like structures) as described[22].

Statistical analysis

Statistics were performed using GraphPad Prism 5 (GraphPad Software) and SPSS 23 (SPSS Inc.). Data are plotted as Tukey box and whisker plots unless stated otherwise. Group sizes were calculated[23] using a relevant and most varied value, previously obtained (plasma IL-6)[6]; expected difference 12 ng/l, spread 10 ng/l, α 0·05, β 0·80. Analyses were carried out on all individuals whenever materials were available and no outliers were excluded.

Results

Experimental infant milk formula transiently lowers body weight gain on high-fat diet

An initially comparable rapid weight gain (PN42–56) preceded a period (PN63–119) of lower weight gain in eIMF compared with cIMF (–9 % on average, $P < 0·05$). From PN126 onwards, weights between groups were no longer significantly different (Fig. 2(a)). Fat and lean mass were similar at PN42. At PN72, 105 and 126, fat mass was substantially lower in eIMF compared with cIMF (–10, –14 and –7 %; $P < 0·01$), and lean mass was slightly lower (–4, –8 and –6 %; $P < 0·01$; Fig. 2(b)), respectively. Fat percentage and lean percentage were similar at PN42. The average fat
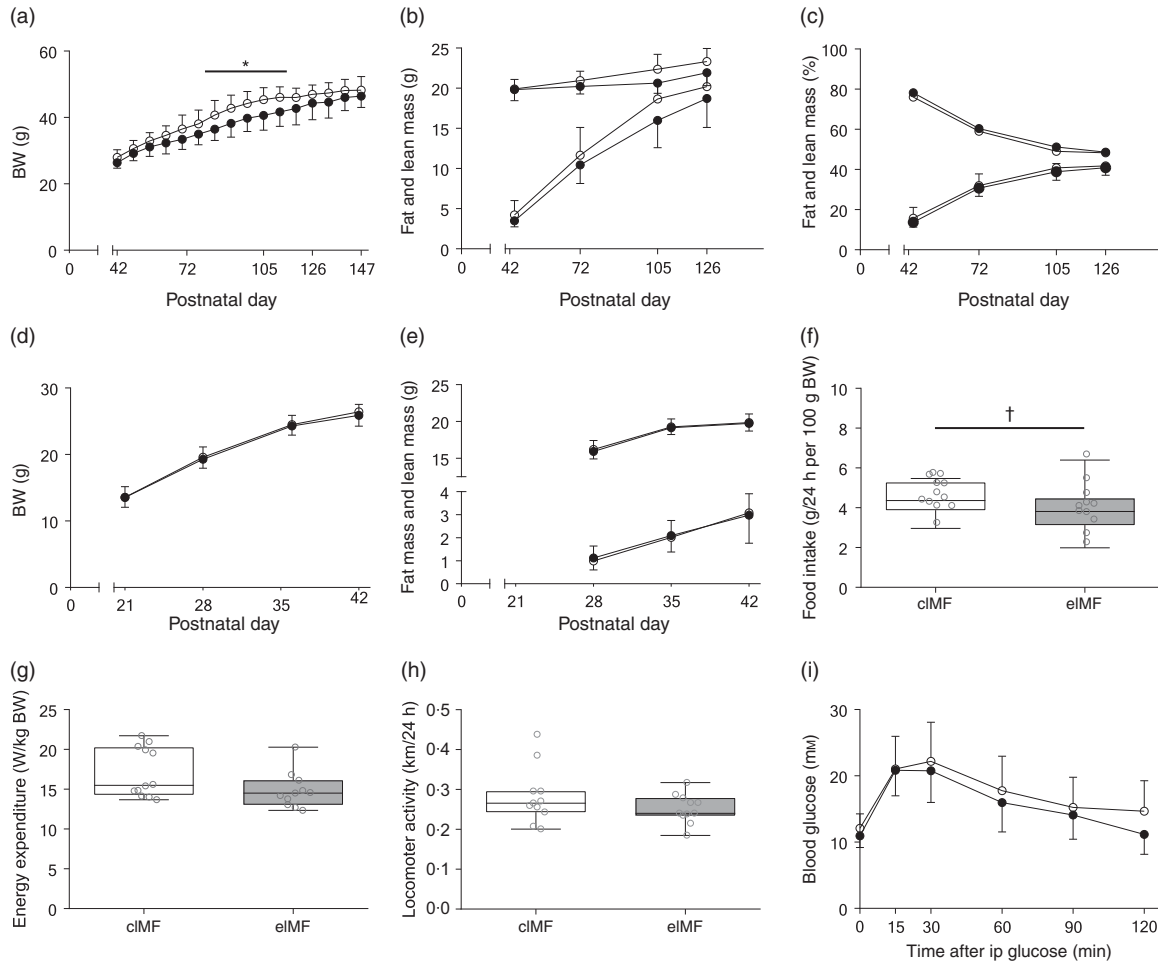
Fig. 2. Mice programmed with experimental infant milk formula (eIMF; –●–) and challenged with a high-fat diet showed a transient lower body weight (BW), lean mass and fat mass compared with animals programmed with control infant milk formula (cIMF; –○–). BW (a), and fat and lean mass (b) are expressed in absolute weights. The percentages of fat and lean mass (c) are expressed as percentage of BW. Early-life BW (d) and fat and lean mass (e) are expressed in absolute weights. Food intake (f), energy expenditure (g) and locomotor activity (h) were measured three times 24 h from postnatal day 154. Glucose tolerance at postnatal day 133 is shown as AUC (i). (a)–(h): n 11–12; (i): n 10–11. Values are means, with standard deviations represented by vertical bars ((a)–(e), (i)). Data are shown as Tukey box plots and scatter plots ((f)–(h)). * $P < 0.05$. † $P < 0.10$. ip, Intraperitoneal.

percentage (PN42–126) was not significantly lower in eIMF (–5 %; $P = 0.07$), whereas lean percentage was not significantly higher in eIMF (+3 %; $P = 0.08$; Fig. 2(c)). To assess whether later life effects were due to differences in growth in early life, we measured body weight from weaning, which was similar between groups, and body composition at PN28 and 35; which was similar. At PN147–154, daily food intake was on average 14 % lower in eIMF ($P = 0.08$, Fig. 2(f)), correlating with the calculated slope of prior weight gain (PN42–PN147, Spearman's rank order, $r_s = 0.6$, $P < 0.01$). Energy expenditure (Fig. 2(g)), and locomotor activity (Fig. 2(h)) were similar. At PN133, the ipGTT time course (Fig. 2(i)) and AUC (2·1 (SD 0·6) *v.* 1·9 (SD 0·4) M × min) was similar between groups. The ipITT (PN140) and ipPTT (PN147) AUC (1·4 (SD 0·3) *v.* 1·2 (SD 0·2) and 1·6 (SD 0·5) *v.* 1·4 (SD 0·4) M × min, respectively) were similar.
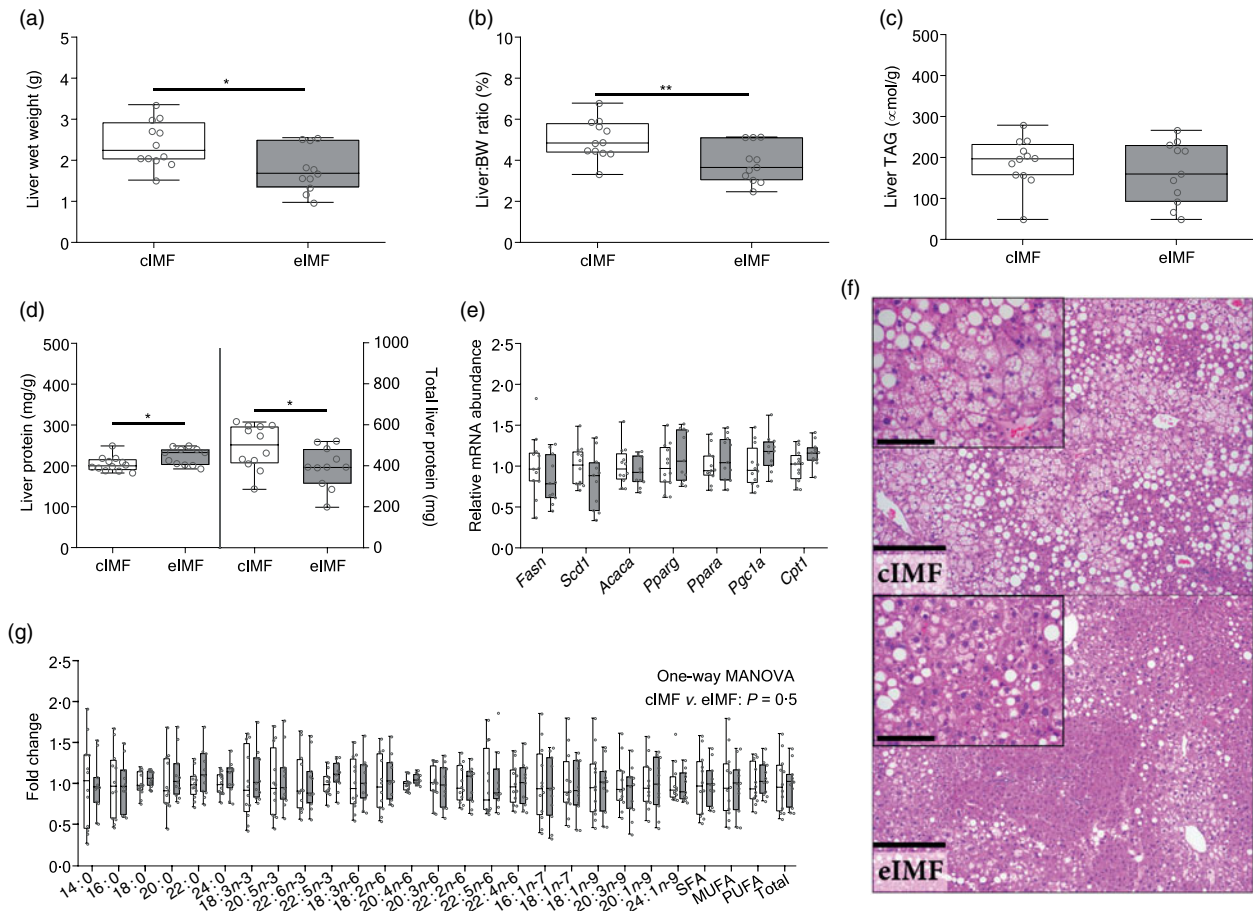
Experimental infant milk formula-programmed mice have a lower liver weight independent of body weight

Upon dissection at PN168, we noted that eIMF-fed mice had lower liver weights (Fig. 3(a); –25 %; $P < 0.05$) and a lower

liver:body weight ratio (Fig. 3(b); –23 %; $P < 0.01$). The lower liver weight was not related to TAG levels, which showed moderate variability (Fig. 3(c)). Liver protein (mg/g liver) was higher in the eIMF group (+9 %; $P < 0.05$), whereas total liver protein was lower in eIMF (mg/liver; –20 %; $P < 0.05$; Fig. 3(d)). Gene expression markers for hepatic *de novo* lipogenesis (fatty acid synthase (*Fasn*), stearoyl-coenzyme A desaturase 1 (*Scd1*), acetyl-coenzyme A carboxylase-α (*Acaca*), peroxisome proliferator activated receptor-γ (*Pparg*)) and fatty acid oxidation (peroxisome proliferator activated receptor-α (*Ppara*), peroxisome proliferative activated receptor-γ, coactivator-1α (*Ppargc1a*, also known as *Pgc1a*), carnitine palmitoyltransferase 1a (*Cpt1*)) (Fig. 3(e)) were similar. The liver fatty acyl-chain profile (Fig. 3(g)) was similar between groups (Fig. 3(g)).

Histological analysis (Fig. 3(h), Table 3) showed that steatosis tended to be higher in cIMF (70 (SD 17) *v.* 57 (SD 32) %, steatosis grade 2·5 (SD 0·5) *v.* 2·1 (SD 1·0)), but this difference did not reach statistical significance. Microvesicular steatosis was more frequent than macrovesicular steatosis in both groups and typically showed a zonal distribution characterised by central

Fig. 3. (Colour online) Mice programmed with experimental infant milk (eIMF; ■) compared with control infant milk formula (cIMF; □) showed a lower liver weight with a concurrent higher protein content without a shift in fatty acyl chain profile. Dissection was performed at postnatal day 168. Liver weight (a) is expressed as wet weight. The liver:body weight (BW) ratio (b) is expressed as percentage of BW. TAG levels (c) are expressed as per g liver. Liver protein content (d) is expressed as mg per g wet liver tissues. Hepatic mRNA levels (e) were normalised to *cyclophilin*. Fatty acyl chain profile is expressed as fold change compared with cIMF (g). Liver histology (haematoxylin and eosin; (f)) showed a zonal distribution characterised by central microvesicular steatosis with mild to moderate mid-zonal macrovesicular steatosis. Bar: 250 μm, inset bar: 100 μm. *n* 11–12. Data are shown as Tukey box plots and scatter plots. * $P < 0.05$, ** $P < 0.01$. *Fasn*, fatty acid synthase; *Scd1*, stearoyl-coenzyme A desaturase 1; *Acaca*, acetyl-coenzyme A carboxylase-α; *Pparg*, peroxisome proliferator activated receptor-γ; *Ppara*, peroxisome proliferator activated receptor-α; *Cpt1*, carnitine palmitoyltransferase 1a; MANOVA, multivariate ANOVA.

microvesicular steatosis with mild to moderate mid-zonal (occasionally extending to portal) macrovesicular steatosis. Lobular inflammation and ballooning were similar between groups. Non-alcoholic fatty liver disease (NAFLD) score, mostly influenced by steatosis, tended to be higher in cIMF but did not reach statistical significance. Varying degrees of biliary/oval cell hyperplasia were seen in almost all mice. The mitotic index (Ki-67) was similar between groups. Binuclear hepatocyte counts in the central and mid/portal region were similar between groups. Hepatic fatty acyl chain ratios representing lipid-related enzymatic activity[24,25] were similar between groups.

Body fat storage was shifted by experimental infant milk formula programming without affecting adult adipokine levels

At PN168, we analysed adipose tissue and plasma adipokines (Fig. 4). Epididymal fat mass was larger in eIMF (Fig. 4(a); +27 %; $P < 0.01$). Inguinal fat mass was similar. Interscapular brown fat mass tended to be smaller in eIMF (−10 %; $P = 0.06$). The median epididymal adipocyte diameter tended

to be higher in eIMF (Fig. 4(b); +11 %; $P = 0.08$). The median perirenal and inguinal adipocyte diameter (Fig. 4(b)) was comparable. Gene expression of peroxisome proliferator activated receptor-γ (*Pparg*), fatty acid synthase (*Fas*), fatty acid binding protein 4 (*Fabp4*), TNF-α (*Tnfa*) and *Cd68* was similar between groups (Fig. 4(c)). The assessed adipokines (leptin, adiponectin, resistin, monocyte chemoattractant protein-1 (MCP-1), TNF-α and IL-6), glucostatic hormones (insulin and glucagon) and cytokines (interferon-γ (Ifnγ), chemokine (C-X-C motif) ligand 1 (Cxcl-1), IL-1β, IL-2, IL-5 and IL-10) were similar (Fig. 4(d)). Crown-like structures in adipose tissue were seen in all cIMF and in 10/11 eIMF mice but tended to occur more often in cIMF (Fig. 4(e); epididymal 8·0 (SD 8·8) *v.* 5·0 (SD 6·9); perirenal 9 (SD 10) *v.* 10 (SD 19)). This difference was not statistically significant.

Discussion

In the present work, we studied the long-term effects of early life exposure (PN16–42) to eIMF *v.* cIMF on body weight and body

Table 3. Hepatic histological scoring and fatty acid ratios of mice programmed with a control infant milk formula (IMF) (*n* 12) or an experimental IMF (*n* 11) and subsequently challenged to a high-fat diet
(Mean values and standard deviations)

		Control IMF		Experimental IMF	
		Mean	SD	Mean	SD
Steatosis	Steatosis grade	2·5	0·5	2·1	1·0
	Steatosis (%)	70	17	57	32
	Location steatosis	12/12 central		8/10 central; 2/10 azonal	
	Hepatocytes with micro- *v.* macrovesicular steatosis (%)	78	9	78	8
	Ballooning	Few, 2/12		Few, 2/11	
	Lobular inflammation	1·0	0·5	1·1	0·6
	Non-alcoholic fatty liver disease score	3·7	0·8	3·3	1·5
	Biliary/oval cell hyperplasia	1·4	0·8	1·5	0·5
Mitosis	Mitotic index (%)	4·3	2·0	4·3	2·7
	Binuclear cells per field (*n*)	4·5	2·1	4·7	3·3
	Central binuclear cells (*n*)	4·7	3·5	4·1	3·6
	Mid/portal binuclear cells (*n*)	4·4	1·7	5·2	3·7
Fatty acyl chain ratios	Σ*n*-6 : Σ*n*-3	6·3	0·6	6·2	0·72
	16 : 1*n*-7/16 : 0	0·13	0·021	0·12	0·037
	18 : 1/18 : 0	8·5	2·6	7·9	2·6
	18 : 1*n*-9/18 : 0	7·8	2·4	7·3	2·4
	18 : 1/16 : 1	13	1·9	14	2·8
	22 : 4*n*-6/18 : 2*n*-6	0·029	0·01	0·025	0·006
	20 : 4*n*-6/20 : 3*n*-6	4·8	1·1	5·3	1·5
	18 : 3*n*-6/18 : 2*n*-6	0·021	0·002	0·020	0·003
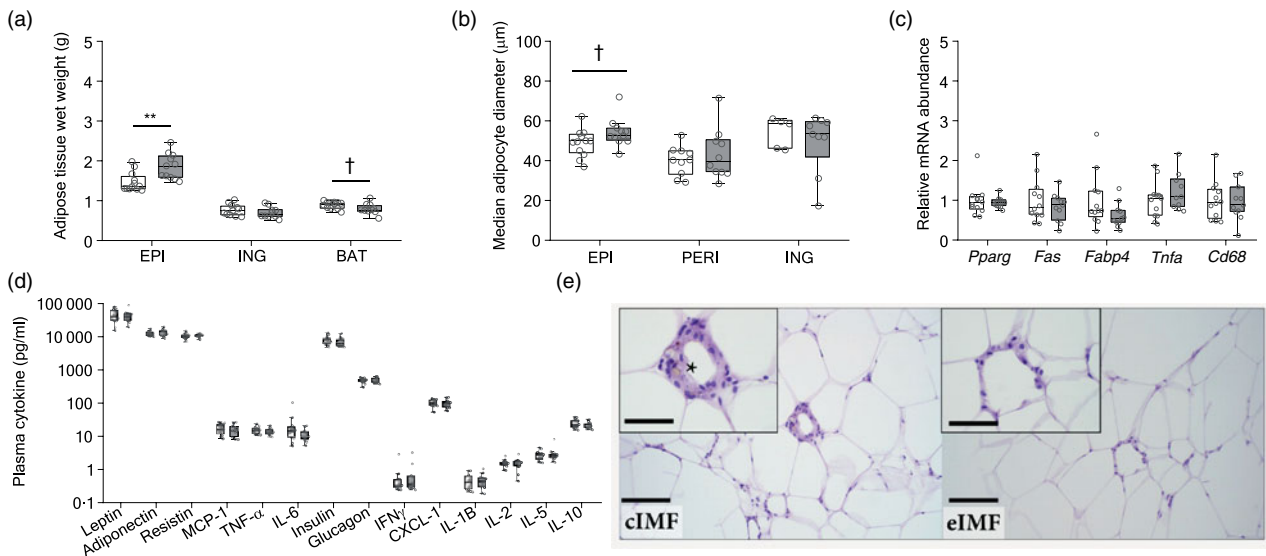
Fig. 4. (Colour online) Mice programmed with experimental infant milk formula (eIMF; ▥) compared with control infant milk formula (cIMF; ▢) showed a higher visceral adipose tissue weight and adipocyte cell diameter. Epididymal (EPI) visceral, inguinal (ING) subcutaneous and interscapular brown adipose tissue (BAT) (a) are expressed as absolute weights. EPI, perirenal (PERI) and ING (b) adipocyte diameter were calculated (Adiposoft) and expressed as equivalent diameter. The epididymal fat gene expression (c) was normalised to *36b4* and shown as fold change. Plasma adipokines, glucostatic hormones and cytokines are expressed as pg/ml (d). Adipose (epididymal depot) pathology (e) characterised by crown-like structures composed of macrophages and other mixed inflammatory cells with lipofuscin (*) surrounding a necrotic adipocyte, bar: 100 μm, inset bar: 50 μm. Data are shown as Tukey box plots and scatter plots (*n* 10–12). * *P* < 0·05, ** *P* < 0·01. † *P* < 0·10. *Pparg*, peroxisome proliferator activated receptor-γ; *Fas*, fatty acid synthase; *Fabp4*, fatty acid binding protein 4; *Tnfa*, TNF-α; *Cd68*, cluster of differentiation 68; MCP-1, monocyte chemoattractant protein-1; IFNγ, interferon-γ; CXCL-1, chemokine (C-X-C motif) ligand 1.

compositional development into adulthood when animals were continuously challenged to a, HFD as well as its effects on size and function of liver and adipose tissues (Fig. 1). Previously, eIMF with a different lipid composition was found to program mice for a lower body fat accumulation when they were challenged with an HFD up to PN126[6]. In the present study, IMF had a different lipid composition and HFD exposure was extended to PN168. Similar programming effects were seen up to PN126, after which differences in body weight and composition disappeared upon continued HFD exposure (Fig. 2). Interestingly, food intake, despite high variability, tended to be lower at PN154 in eIMF-fed mice (Fig. 2), strongly correlating with the slope of prior weight gain. This suggests that the differences in body weight can, at least in part, be attributed

to differences in food intake. The transient effects observed may indicate that the programming response, that is, the initial lower fat accumulation in adipose stores, can be overruled by a strong and persistent dietary challenge. In addition, our study suggests these programming effects, due to early life eIMF exposure, occurred regardless of the lipid composition of the fat globules and is rather caused by the physicochemical structure of the lipids, that is, globule size and phospholipid coating[10]. In humans and mammals, the amount of lipids in (mature) milk, despite highly variable diets, is remarkably stable[26,27]. The size of milk fat globules, as well as the milk TAG content, seems to be tightly regulated and species specific[28] and greatly impacts the absorption kinetics of breast milk lipids[29,30]. Testing eIMF (large, phospholipid-coated fat globules) *v.* cIMF (small, uncoated fat globules) in adult men resulted in a different postprandial response upon a single bolus intake: an earlier postprandial glucose and insulin time to peak, an earlier NEFA time to nadir and a later cholecystokinin time course[30]. We hence speculate that fat globule size and phospholipid coating program metabolic and tissue development induced by differential lipid uptake kinetics or postabsorptive lipid trafficking or tissue partitioning. Lipid uptake kinetics of eIMF, compared with cIMF, are likely to be more comparable to breast milk.

Early life feeding with eIMF compared with cIMF lowered liver weight in later life, independent of body weight (Fig. 3). The difference was not explained by mitotic index or the degree of polyploidisation. Hepatic TAG content had moderate variability without clear correlation with other parameters. Possibly the early life diet primed hepatic tissue for a different response to an HFD challenge, resulting in a differential tissue growth. It is also possible, however, that the transient difference in body weight (or adipose tissue development) triggers a difference in liver size. Previously, a non-significantly higher liver weight had been seen in cIMF compared with eIMF and to an unchallenged control group[9]. Additionally, a higher liver weight was seen upon early life feeding with an IMF containing small compared with large lipid globules and challenging with an HFD (10). We hypothesise that PN liver development was changed due to a difference in post-absorptive lipid handling and trafficking. As mentioned earlier, eIMF is more rapidly absorbed compared with cIMF in adult men[30]. In addition, a gavage of breast milk, compared with standard formula, leads to a more rapid chylomicron (CM) production and a more rapid absorption of palmitic, arachidonic and DHA in adult rats[29]. Rapid absorption of protein-coated fat results in three-fold larger CM diameter[31]. However, we believe rapid lipid absorption only leads to larger CM when enteral phospholipid supply is limited, necessitating higher volume:surface area ratios[32]. In contrast, CM diameter is lowered by biliary phospholipids[32]. It is possible that breast milk, and likewise eIMF, is rapidly absorbed and produces smaller CM compared with cIMF due to the dietary phospholipids provided with breast milk and eIMF. The observation that an IMF with large globules but lacking phospholipids does not program mice for less fat accrual in later life[10], adding weight to this notion. CM size and number affect the plasma half-life, as smaller particles have a larger relative surface area available to enzymes[33,34], and are more quickly removed from the plasma

via liver sieving[35]. CM produced upon eIMF or human milk ingestion are expected to have a different fractional clearance rate compared with CM upon cIMF ingestion. It is tempting to speculate that a more rapid absorption plus utilisation, opposed to storage, of breast milk and eIMF-derived lipids in early life programs metabolism and fat accumulation capacity towards an advantageous trait for later life health.

The effect on fat pad weights and adipocyte diameter was independent of body weight and composition (Fig. 4). Previous studies showed that at PN98 the epididymal fat pad was smaller in eIMF-fed mice[9,10], whereas this difference between test groups had disappeared at PN126[6]. We observed a higher epididymal fat mass in eIMF at PN168, indicating a differential fat distribution in eIMF *v.* cIMF, as the perirenal and inguinal fat pads were similar in mass. Counterintuitively, the larger epididymal fat pads seen in eIMF did not result in lower levels of adiponectin but did result in higher levels of inflammatory markers (TNF-α, IL-6), MCP-1 or resistin (Fig. 4) as typically seen with larger visceral adipose tissue[36]. Interestingly, we found no effect on glucose metabolism and homoeostasis (Fig. 2(i)). Previously, eIMF-fed mice had lower plasma leptin, resistin, glucose and homoeostatic model assessment of insulin resistance at PN126(6), likely related to the lower fat mass. A lower fat accrual rate, transiently seen in eIMF, is advantageous to metabolic health. However, given the observed minor effects on adipose tissue at PN168, we think it is unlikely that adipose tissue initiates the programming effect and merely is a logical consequence and trait of the programmed phenotype.

Conclusion

The present study shows that feeding a PN diet containing large phospholipid-coated lipid globules has transient effects on body fat accrual during prolonged exposure to HFD. These effects are limited in strength and robustness and can be overruled by (too) strong environmental features, such as continued HFD feeding. Our findings indicate the robustness and the limits of early life programming due to eIMF exposure in the mouse model used to study. The observed programming effects are hypothesised to be due to a difference in fat absorption and/or post-absorptive handling and trafficking in the body.

Acknowledgements

Supplementary material

To view supplementary material for this article, please visit https://doi.org/10.1017/S0007114519002083

References

1. Arenz S, Ruckerl R, Koletzko B, et al. (2004) Breast-feeding and childhood obesity – a systematic review. Int J Obes Relat Metab Disord 28, 1247–1256.
2. Owen CG, Whincup PH, Odoki K, et al. (2002) Infant feeding and blood cholesterol: a study in adolescents and a systematic review. Pediatrics 110, 597–608.
3. Owen CG, Whincup PH, Gilg JA, et al. (2003) Effect of breast feeding in infancy on blood pressure in later life: systematic review and meta-analysis. BMJ 327, 1189–1195.
4. Gallier S, Vocking K, Post JA, et al. (2015) A novel infant milk formula concept: mimicking the human milk fat globule structure. Colloids Surf B Biointerfaces 136, 329–39.
5. Armand M, Hamosh M, Mehta NR, et al. (1996) Effect of human milk or formula on gastric function and fat digestion in the premature infant. Pediatr Res 40, 429.
6. Oosting A, Kegler D, Wopereis HJ, et al. (2012) Size and phospholipid coating of lipid droplets in the diet of young mice modify body fat accumulation in adulthood. Pediatr Res 72, 362–369.
7. Bourlieu C, Menard O, De La Chevasnerie A, et al. (2015) The structure of infant formulas impacts their lipolysis, proteolysis and disintegration during in vitro gastric digestion. Food Chem 182, 224–235.
8. Bourlieu C & Michalski MC (2015) Structure–function relationship of the milk fat globule. Curr Opin Clin Nutr Metab Care 18, 118–227.
9. Oosting A, van Vlies N, Kegler D, et al. (2014) Effect of dietary lipid structure in early postnatal life on mouse adipose tissue development and function in adulthood. Br J Nutr 111, 215–226.
10. Baars A, Oosting A, Engels E, et al. (2016) Milk fat globule membrane coating of large lipid droplets in the diet of young mice prevents body fat accumulation in adulthood. Br J Nutr 115, 1930–1937.
11. Pettersson US, Walden TB, Carlsson PO, et al. (2012) Female mice are protected against high-fat-diet induced metabolic syndrome and increase the regulatory T cell population in adipose tissue. PLOS ONE 7, e46057.
12. Reeves PG, Nielsen FH & Fahey GC Jr (1993) AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr 123, 1939–1951.
13. Cariou B, van Harmelen K, Duran-Sandoval D, et al. (2006) The farnesoid X receptor modulates adiposity and peripheral insulin sensitivity in mice. J Biol Chem 281, 11039–11049.
14. Doktorova M, Zwarts I, Zutphen TV, et al. (2017) Intestinal PPARdelta protects against diet-induced obesity, insulin resistance and dyslipidemia. Sci Rep 7, 846.
15. Verkade HJ, Hoving EB, Muskiet FA, et al. (1991) Fat absorption in neonates: comparison of long-chain-fatty-acid and triglyceride compositions of formula, feces, and blood. Am J Clin Nutr 53, 643–651.
16. Dimova LG, de Boer JF, Plantinga J, et al. (2017) Inhibiting cholesterol absorption during lactation programs future intestinal absorption of cholesterol in adult mice. Gastroenterology 153, 382–385.e3.
17. Kleiner DE, Brunt EM, Van Natta M, et al. (2005) Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 41, 1313–1321.
18. Lackner C (2011) Hepatocellular ballooning in nonalcoholic steatohepatitis: the pathologist's perspective. Expert Rev Gastroenterol Hepatol 5, 223–231.
19. Sutton ME, op den Dries S, Koster MH, et al. (2012) Regeneration of human extrahepatic biliary epithelium: the peribiliary glands as progenitor cell compartment. Liver Int 32, 554–559.
20. Gentric G, Maillet V, Paradis V, et al. (2015) Oxidative stress promotes pathologic polyploidization in nonalcoholic fatty liver disease. J Clin Invest 125, 981–992.
21. Galarraga M, Campion J, Munoz-Barrutia A, et al. (2012) Adiposoft: automated software for the analysis of white adipose tissue cellularity in histological sections. J Lip Res 53, 2791–2796.
22. Greaves P, Chouinard L, Ernst H, et al. (2013) Proliferative and non-proliferative lesions of the rat and mouse soft tissue, skeletal muscle and mesothelium. J Toxicol Pathol 26, Suppl. 3, 1s–26s.
23. Rosner B (2015) Fundamentals of Biostatistics. Toronto: Nelson Education.
24. Kotronen A, Seppanen-Laakso T, Westerbacka J, et al. (2010) Comparison of lipid and fatty acid composition of the liver, subcutaneous and intra-abdominal adipose tissue, and serum. Obesity (Silver Spring) 18, 937–944.
25. Jump DB (2011) Fatty acid regulation of hepatic lipid metabolism. Curr Opin Clin Nutr Metab Care 14, 115–120.
26. Finley DA, Lonnerdal B, Dewey KG & Grivetti LE (1985) Breast milk composition: fat content and fatty acid composition in vegetarians and non-vegetarians. Am J Clin Nutr 41, 787–800.
27. Kelishadi R, Hadi B, Iranpour R, et al. (2012) A study on lipid content and fatty acid of breast milk and its association with mother's diet composition. J Res Med Sci 17, 824–827.
28. Gantner V, Mijić P, Baban M, et al. (2015) The overall and fat composition of milk of various species. Mljekarstvo/Dairy 65, 223–231.
29. Vurma M, DeMichele S & Tso P (2018) Comparison of fat absorption mechanisms in vivo between human milk and infant formula containing novel absorption enhancement technology. In 51st ESPGHAN Annual Meeting, Geneva, Switzerland 2018.
30. Baumgartner S, van de Heijning BJM, Acton D & Mensink RP. (2017) Infant milk fat droplet size and coating affect postprandial responses in healthy adult men: a proof-of-concept study. Eur J Clin Nutr 71, 1108–1113.
31. Vors C, Pineau G, Gabert L, et al. (2013) Modulating absorption and postprandial handling of dietary fatty acids by structuring fat in the meal: a randomized crossover clinical trial. Am J Clin Nutr 97, 23–36.
32. Werner A, Havinga R, Perton F, et al. (2006) Lymphatic chylomicron size is inversely related to biliary phospholipid secretion in mice. Am J Physiol Gastrointest Liver Physiol 290, G1177–G1185.
33. Goldberg IJ (1996) Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis. J Lipid Res 37, 693–707.
34. Martins I, Mortimer B, Miller J & Redgrave T (1996) Effects of particle size and number on the plasma clearance of chylomicrons and remnants. J Lipid Res 37, 2696–2705.
35. Braet F & Wisse E (2002) Structural and functional aspects of liver sinusoidal endothelial cell fenestrae: a review. Comp Hepatol 1, 1.
36. Hamdy O, Porramatikul S & Al-Ozairi E (2006) Metabolic obesity: the paradox between visceral and subcutaneous fat. Curr Diabetes Rev 2, 367–373.