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Long-term consequences of under-nutrition during suckling on glucose tolerance and lipoprotein profile in female and male rats

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To determine the effect of under-nutrition during suckling in adults, at delivery female Sprague Dawley rats were allowed to lactate litters of either eight (controls) or sixteen pups each (large litter, LL). The amount of milk taken by LL pups was less than the controls and the concentration of triacylglycerols (TG) in the milk of the former was lower. The increase of both body weight and length in LL was lower than in the controls during suckling. At weaning, pups were allowed to eat *ad libitum* a standard diet and whereas at 20 months female body weight did not differ between LL and control rats, LL males weighed less than controls. Plasma NEFA were lower in male LL than in controls at 10 months, leptin at 10 and 16 months and TG and VLDL-TG at 20 months, with no differences in females. When 20 months old, lumbar and epididymal adipose tissue weights were lower in male LL than in controls, but not in females. The increase in plasma insulin after oral glucose load was lower in LL than in controls, both in males and females at 4 and 16 months, and only in males at 10 months, whereas the change in plasma glucose remained constant between the groups. Results indicate that both the pancreatic β-cell function and insulin sensitivity and adipose tissue metabolism are independently programmed as a consequence of under-nutrition during suckling, the effect being more manifest for males than for females.

Programming: Gender: Litter size: Adipose tissue: Insulin: Lipoproteins

The most prevalent form of nutritional disorder of children in developing countries is malnutrition, whereas in adults overweight is the most widespread. Epidemiological studies have linked low birth weight with impaired glucose tolerance, abnormalities in blood lipid concentrations and risk for CVD and obesity in adults (Ravelli *et al.* 1976; Lucas, 1991; Barker, 1995; Leon, 1998; Waterland & Garza, 1999; Lauren & the EURO-BLCS Study Group, 2003). These alterations have been attributed to a mechanism whereby at critical periods of fetal and early development certain metabolic processes are programmed in the organism to accommodate a particular level of nutrition throughout its life, thus providing a survival advantage (Lucas, 1991; Hales & Barker, 1992). However, subsequent improvements in nutrition during adult life appear to be a major cause for these pathophysiological alterations.

In view of the shorter life span of animals and the possibility of controlling the genetic and environmental influences, several animal models of developmental programming have been applied to understand the specific mechanisms responsible for these phenomena (Armitage *et al.* 2004). Although studies in rats support the hypothesis that prenatal and early postnatal nutrition permanently affect glucose–insulin relationships (Passos *et al.* 2000;Waterland & Garza, 2002), additional data are needed to determine whether long-term effects of perinatal malnutrition modifying the glucose–insulin relationship are also associated with altered lipid metabolism, which by itself could affect glucose tolerance and insulin sensitivity. This is, however, a necessary step towards understanding the significance of altered nutrition during the perinatal phase in human health and designing potential interventions to avoid or ameliorate their adverse consequences. Several strategies have been used to determine the response in restricting fetal and/or early postnatal growth as a consequence of transient nutritional conditions early in life. One strategy is the reduction of the protein content of the maternal diet during pregnancy, where a transitory period of increased insulin sensitivity, followed by no change and an impaired glucose tolerance with frank diabetes was found, depending on the age of the animals studied (Hales & Ozanne, 2003). Changes in the quality of nutrition, without affecting the total energy intake during the immediate postnatal period, have also been shown to cause metabolic programming. An increase in carbohydrate-derived energy during suckling caused a sustained hyperinsulinaemia in adulthood without dietary intervention (Vadlamudi *et al.* 1993) and formed the basis for adult-onset obesity (Vadlamudi *et al.* 1995; Srinivasan *et al.* 2003). Another strategy was to adjust the litter size during lactation so that pups in small litters received more milk than those in larger litters. This was found to cause lifetime growth alterations (McCance, 1962; Plagemann *et al.* 1992) and both large and small litters were determined to have reduced pancreatic β-cell glucose-responsiveness when adults (Waterland & Garza, 2002).

Abbreviations: AUC, area under the curve; LL, large litter; Ch, cholesterol; TG, triacylglycerol.

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Under-nutrition during the perinatal period could have long-term effects not only in the glucose and insulin axis but also in the level of circulating lipids in the offspring, but these two specific metabolic sites have not been previously evaluated in the same animals at different ages after food restriction during the suckling period, nor determining the differences in the gender response. The present study was therefore addressed to analyse these two metabolic sites at different ages in the same male and female animals after postnatal malnutrition in order to determine the potential interaction between them. Thus, by increasing litter size in rats during lactation we determined the long-term consequences of postnatal malnutrition on glucose-tolerance tests, plasma lipids and plasma lipoprotein profile.

Materials and methods

Female Sprague-Dawley rats from our animal quarters were housed under controlled light and temperature conditions (12 h light–dark cycle; 22 ± 1°C). Rats were mated when they weighed 180–190 g and fed *ad libitum* a purified diet containing the following per kg: 170 g casein; 100 g cellulose; 580 g maize starch; 100 g olive oil; 35 g salt mix; 10·1 g vitamin mix. Other details of this diet composition and its characteristics have been previously reported (Amusquivar *et al.* 2000) and it was considered suitable as a control diet. The experimental protocol was approved by the Animal Research Committee of the University San Pablo-CEU. After delivery (24 h), pups from the different dams were pooled and distributed at random in two groups, one of lactating dams with litters of eight pups each (controls) and the other one of lactating dams with litters of sixteen pups each (large litters, LL). They were maintained under these conditions until weaning (at 21 d). From this time, female and male pups were separated in different cages and all the animals were fed *ad libitum* a standard non-purified diet (B&K Universal, Barcelona, Spain). Milk yield was estimated from pup weight and weight gain on days 7–8 and days 15–16 of lactation, as previously described (Sampson & Jansen, 1984). On day 10 of lactation, after being separated from their litters, some dams were anaesthetized with a cocktail (0·5 ml/200 g body weight) containing 9 mg ketamine (Imalgene 500; Rhone Merieux, Lyon, France) and 0·25 mg chlorpromazine (Largactil; Rhone Poulenc, Madrid, Spain) administered intraperitoneally. They were injected intraperitoneally with a solution of oxytocin (0·25 ml/200 g body weight: 40 mg/l Syntocinón; Novartis Farmaceutica, Barcelona, Spain) and milk was obtained with gentle hand stripping of the teats. An aliquot of milk was immediately placed into chloroform–methanol (2:1, v/v) for lipid extraction (Folch *et al.* 1957) and once phospholipids had been eliminated with alumina in isopropylalcohol, samples were processed for the analysis of triacylglycerols (TG) (Sigma, St. Louis, MO, USA). Some rats from each group were decapitated at different ages and trunk blood collected into receptacles containing 1 g Na_2-EDTA/l for plasma separation. Plasma was kept at − 80°C until analysed for glucose, TG, NEFA and immunoreactive insulin by commercial kits (Boehringer-Mannheim, Mannheim, Germany, Menarini Diagnostic, Florence Italy, Wako Chemical GmbH, Neuss, Germany and Mercodia AB, Uppsala, Sweden, respectively). Leptin was assayed by ELISA using a commercial kit specific for rat (DRG Diagnostics, Mountainside, NJ, USA). Liver, brain and periuterine, epididymal and lumbar adipose tissue were immediately dissected and weighed and aliquots of liver tissue were placed into liquid N and kept at − 80°C until analysis. Frozen liver aliquots were used for lipid extraction (Folch *et al.* 1957) and aliquots of lipid extracts were quantified for different lipid moieties after image analysis and separation by one-dimensional TLC (Ruiz & Ochoa, 1997) using the G5–700 BIOIMAGE TLC scanner of Bio-Rad (Hercules, CA, USA). Spots were quantified as integrated optical densities against an internal standard of cholesterol formate and calibration curves of TG, cholesterol (Ch), esterified-Ch and fatty acids standards.

Oral glucose tolerance tests were performed as follows. All tests were conducted between 09.00 and 12.00 hours in overnight fasted rats. After collecting tail blood (0 time), rats received an oral load of 2 g glucose/kg body weight and blood was collected from the tail at 7·5, 15, 22·5, 30 and 60 min and processed for plasma glucose and insulin determinations as earlier. Blood samples obtained at 0 time were also used for the analysis of plasma TG and NEFA, as earlier.

In rats killed at 20 months, plasma was used for VLDL separation by ultracentrifugation at 224 000 ***g*** for 3 h at density 1·006 kg/l in a Beckman TL-100 ultracentrifuge (Beckman Instruments, Madrid, Spain) with a Beckman TLA 100·2 rotor, as previously described (Munilla & Herrera, 1997). Supernatant fractions were recovered by tube slicing whereas the remaining apoB-100 containing lipoproteins (mainly LDL) were precipitated in the infranatants by treatment with a mixture (10:1, by vol.) of 14 mM-phosphotungstic acid and 2 mM-$MgCl_2$. After sitting for 24 h at 4°C, HDL were separated in the supernatant after centrifugation at 15 000 rpm for 5 min. TG and Ch concentrations were determined by enzymatic methods with commercial kits (Menarini Diagnostics).

Statistics

All analyses were conducted in SPSS (version 14.0; Chicago, IL, USA). Results were expressed as means with their standard errors. ANOVA with repeated measures with Bonferroni correction for multiple comparisons was used to compare the group differences in plasma lipids, leptin levels and glucose tolerance tests at different ages. Differences between means were determined by two-way ANOVA with litter size and gender as the fixed factors; significant interactions by litter size or gender were then analysed by Student's *t* test. Data where the variances were not equal were log-transformed prior to analysis. Differences between the two groups at 20 d old were analysed by Student's *t* test.

Results

Body weight and length and metabolic variables during suckling

Whereas on the first day of age, body weight and body length of pups from LL (sixteen pups per litter) did not differ from controls (eight pups per litter), from day 5 until weaning (day 21) LL pups weighed less and were smaller than those from controls, the difference in both variables increasing as the pups got older (Fig. 1). Although total milk yield expressed as g per d was higher in dams with LL than controls, pups in the first group received significantly less amounts of milk per d than those in

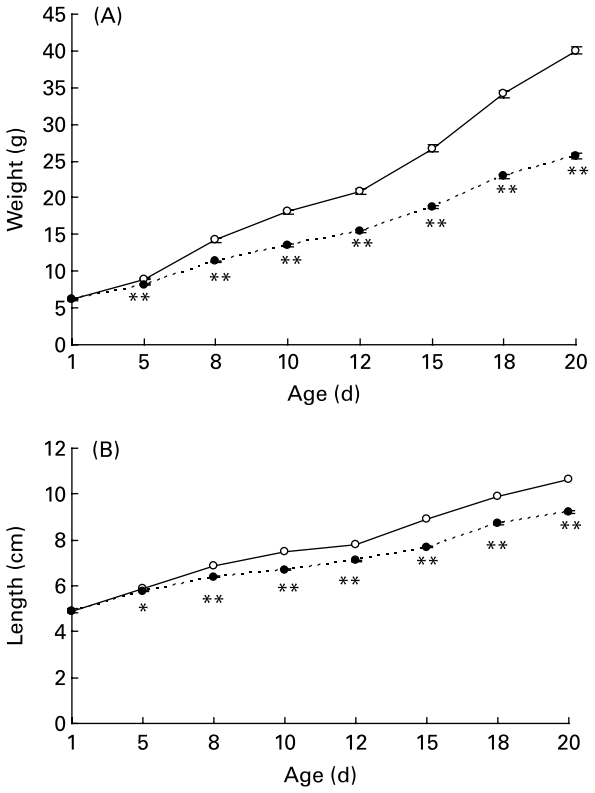

Fig. 1. Body weights (A) and lengths (B) during suckling in control (– ○ –) and large litter (LL; – • –) sizes. Values are means with their standard errors for ten to twenty-seven representative pups from each litter (day 1) and total number of pups (fifty-six to ninety-four; days 5–20). Mean values were significantly different between the two groups on the corresponding days: *$P<0{\cdot}05$; **$P<0{\cdot}01$. For details of animals and procedures, see p. 1031.

the second at both day 8 (2·79 (SEM 0·15) g/pup per d in controls and 1·70 (SEM 0·05) in LL; $P<0{\cdot}001$) and day 15 of lactation (4·01 (SEM 0·19) in controls and 2·56 (SEM 0·11) in LL; $P<0{\cdot}001$). Milk TG concentration was also lower in dams of LL than in those of controls (552 (SEM 14) μmol/ml in controls and 380 (SEM 36) in LL, $P<0{\cdot}01$).

At the end of the suckling period (day 20) lower body weight from LL rats was also accompanied by lighter liver and brain weights as well as lower plasma glucose, insulin, leptin and TG levels, with no difference in plasma NEFA and Ch levels nor in liver TG concentration compared with controls (Table 1).

Body weight and metabolic variables at different ages after weaning

Body weight in males progressively increased until 16 months, values being consistently lower in those from LL than from control rats (Fig. 2). A similar trend was also found in females and, although absolute values were always lower in females, body weight in female rats of the LL group was lower than in controls until age 16 months (Fig. 2). Plasma levels of TG in male rats were not significantly lower in LL rats at 4, 10 or 16 months (Table 2). In females, plasma TG levels did not differ between LL and control groups at 4, 10 and 16 months old, although there was also a tendency for them to be lower in LL than in controls. Plasma TG levels were lower in female than in male control rats at 10 months, and values at 16 months were always higher in both males and females as when the animals were younger. Plasma NEFA levels in males were lower in LL rats than in controls when they were 10 months old, the difference disappearing when they were 4 or 16 months old (Table 2). Unlike those found in males, plasma NEFA levels in females did not differ between the two groups at any of the time points studied, although values at 16 months were higher than in males. In males, there were no differences in plasma Ch levels between the two groups at 4 and 10 months old, but they were significantly lower in the LL group than in the controls at 16 months, whereas in females no difference between the two groups was found at any of the time points studied (Table 2). Plasma leptin levels were measured only in 10 and 16 month old males and in both cases values were significantly lower in those of the LL group than in controls (Table 2). In females, plasma leptin levels were measured only at 16 months, and although values were lower in LL than in control animals, the difference did not reach statistical significance.

Animals were killed at 20 months. At this age, body weight and size were lower in male LL than in male controls. This difference was not observed in females, the respective values of which were lower than in males (Table 3). Furthermore, in male liver, kidney, lumbar adipose tissue and epididymal fat pads, weights were lower in LL rats than in

Table 1. Tissue weights and metabolic variables in 20-d-old rats suckled in control and large litter (LL) sizes*
(Values are means with their standard errors for six to seven rats per group)

	Control		LL		
	Mean	SEM	Mean	SEM	Statistical significance *P*
Liver weight (g)	1·43	0·09	0·85	0·06	0·000
Brain weight (g)	1·31	0·03	1·20	0·04	0·043
Plasma glucose (mg/l)	1581	28	1465	17	0·006
Plasma insulin (pM/ml)	90·6	19·8	35·4	14·4	0·049
Plasma leptin (ng/ml)	44·0	5·6	19·9	1·5	0·001
Plasma TG (mg/l)	2214	140	1503	169	0·007
Plasma NEFA (μM)	551·9	13·8	380·4	35·7	NS
Plasma cholesterol (mg/l)	1863	22	1959	153	NS
Liver TG (μmol/g tissue)	67·4	2·2	59·8	15·3	NS

* For details of animals and procedures, see p. 1031.
TG, triacylglycerol.

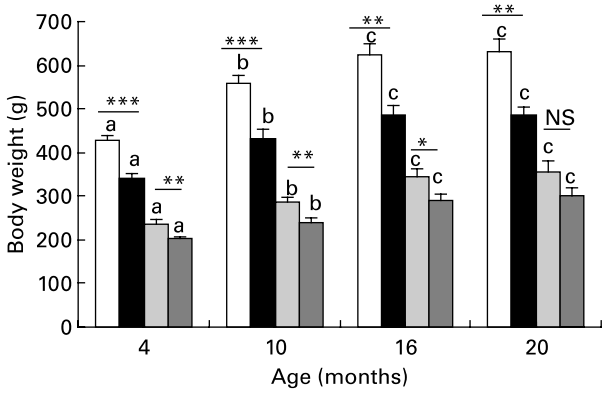

Fig. 2. Body weight in male control (□) and large litter (■) and female (control ▫ and large litter ▪) size rats at different ages after weaning. Values are means with their standard errors for seven to twelve rats per group. [a,b,c] Mean values with unlike superscript letters were significantly different and indicate statistical comparison over the entire study period ($P<0·05$). Mean values were significantly different between control and LL rats: *$P<0·05$; **$P<0·01$; ***$P<0·001$.

controls. These differences disappeared for liver and kidney weights when corrected by body weight, but they were maintained statistically lower for male LL than control rats in the case of lumbar adipose tissue and epididymal fat pads (Tables 3 and 4). The organ weights of 20-month-old females were lower than in males and they were similar for LL and control groups, the only significant difference being for kidney weight, which was lower in the LL group. No significant difference was found in absolute values when corrected by body weight for liver, kidney, lumbar adipose tissue or periuterine adipose tissue (Tables 3 and 4).

A lipoprotein profile was obtained in the 20-month-old rats. In males, plasma TG levels were lower in LL than in controls, this change mainly corresponding to those of VLDL, whose levels were lower in LL than in control groups, with no difference between the two groups in HDL-TG (Table 5). In males, plasma Ch levels were lower in 20-month-old LL rats than in controls, the difference mainly corresponding to LDL-Ch, with no differences in VLDL- or HDL-Ch. In 20-month-old females, no difference between LL and control groups was found in plasma TG, VLDL-TG or HDL-TG (Table 5). However, plasma Ch levels were higher in LL than in control female rats, this difference also appearing in both HDL- and LDL-Ch, with no difference in VLDL-Ch (Table 5).

A different trend between 20-month-old males and females was found in the distribution of liver lipid concentration. Whereas in males, the only change between LL and control rats appeared in a lower TG concentration in the former group, decreased concentrations of both Ch and esterified-Ch and an enhanced fatty acid concentration were found in liver of LL female rats compared with controls (Table 6).

Oral glucose tolerance tests

Basal plasma glucose and insulin levels (0 time) did not differ between LL and control rats at 4, 10 or 16 months (data not shown). However, major differences were found as a response to the oral glucose load. Values are expressed as the area under the curve (AUC) for both glucose and insulin during the 60 min of the test. It was found that the AUC for glucose in males at the age of 4, 10 and 16 months was very stable and did not differ between LL and control rats (Table 7). However, the AUC for insulin in these same males progressively increased as they became older, values always being significantly lower in LL than in control rats (Table 7). In females, the AUC of glucose was very similar to that observed in males and it too was very stable with age, with no difference between LL and control rats. The AUC values for insulin in females tended to be consistently lower than those found in males. In female control rats, the AUC values for insulin did not increase with age, whereas in LL female rats, with the exception of 10-month-old females, values were significantly lower than those observed in the control group (Table 7).

Discussion

The present study consistently shows that under-nutrition during the suckling period due to LL size in rats causes a permanent reduction in glucose-stimulated insulin secretion despite a normal glucose tolerance, suggesting an enhanced insulin sensitivity. Although this finding may be related to the hypolipidaemia tendency in males, this is not, however, the case in females, suggesting that the endocrine pancreas was primarily programmed as result of the under-nutrition during suckling.

During suckling the reduced milk intake in LL rats is added to a reduction in TG concentration in milk, which is the most abundant lipid component in milk (Vilaró *et al.* 1987; Barbas & Herrera, 1998) and therefore the main energy source for the suckling newborn. Other under-nutrition conditions such as that caused by a glucose-restricted diet during lactation have also been shown to decrease milk lipid concentration in rats (Lanoue & Koski, 1994). This condition of decreased milk intake and lower milk TG content causes metabolic and endocrine disturbances in the suckling newborns, as shown here by decreased plasma glucose, TG, insulin and leptin concentrations. The leptin concentration is probably related to a decreased adipose tissue mass, as it coincides with the lower body weight of the pups, which was more noticeable than the reduced length of the LL animals. A blunted insulin secretion after a glucose stimulus and a lack of capacity to recover normal fat depots when LL animals are allowed to eat *ad libitum* standard rat chow from weaning seems to be programmed by the under-nutrition condition during suckling. The decreased β-cell response in LL rats when adults, confirms previous findings (Waterland & Garza, 2002) and supports the hypothesis that the endocrine pancreas contributes to the programming in this model. Since the LL rats consistently exhibited normal glucose tolerance, the findings suggest increasing insulin sensitivity, which was maintained until the animals were 16 months old, when the last oral glucose tolerance test was performed, and corresponds to middle-age for the Sprague Dawley rat (Bassant *et al.* 1994; Barzilai & Rossetti, 1996). Although this effect could be partially related to the hypolipidaemia indirectly derived from the decreased fat depot of the studied animals, this condition was only observed in males in the form of decreases in plasma NEFA levels at 10 months, and could result from a decreased lipolytic activity secondary to the enhanced insulin sensitivity. It was not observed in females, where the decreased insulin release after the oral glucose load was also impaired in the LL

Table 2. Plasma lipid and leptin levels at different ages in rats suckled in control and large litter (LL) sizes‡
(Values are means with their standard errors for six to twelve rats per group)

Age (months)	4		10		16	
	Mean	SEM	Mean	SEM	Mean	SEM
Triacylglycerol (mg/l)						
Male						
Control	958[a]	138	1110[a]	83	1833[b]	240
LL	685[a]	112	893[ab]	104	1365[b]	152
Female						
Control	638[a]	73	630[a]††	46	1687[b]	276
LL	494[a]	71	668[ab]	59	1439[b]	187
Significance of effect of:						
Gender	0·031		0·000		NS	
Litter size	0·048		NS		NS	
Litter size × Gender	NS		NS		NS	
NEFA (μM)						
Male						
Control	1241[ab]	61	1391[a]	27	1206[b]	36
LL	1265[a]	108	1204[a]*	60	1220[a]	60
Female						
Control	1129[a]	99	1313[a]	38	1592[a]†	122
LL	1048[a]	82	1276[ab]	33	1576[b]††	97
Significance of effect of:						
Gender	NS		NS		0·000	
Litter size	NS		0·014		NS	
Litter size × Gender	NS		NS		NS	
Cholesterol (mg/l)						
Male						
Control	769[a]	51	1120[b]	51	1283[c]	87
LL	775[a]	34	920[b]	53	905[ab]**	67
Female						
Control	840[a]	76	1023[ab]	35	1127[b]	92
LL	923[a]	57	1024[a]	49	1023[a]	74
Significance of effect of:						
Gender	NS		NS		NS	
Litter size	NS		NS		0·010	
Litter size × Gender	NS		NS		NS	
Leptin (ng/ml)						
Male						
Control			39·4[a]	3·9	81·8[a]	18·4
LL			19·6[a]**	1·0	29·0[a]*	6·3
Female						
Control	ND				52·0	8·3
LL	ND				30·6	6·9
Significance of effect of:						
Gender					NS	
Litter size	0·005				0·004	
Litter size × Gender					NS	

[a,b,c] Mean values with unlike superscript letters within a row were significantly different and indicate statistical comparison over the entire study period ($P<0·05$).
Mean values were significantly different from those for the control rats: *$P<0·05$; **$P<0·01$.
Mean values were significantly different from those for the male rats: †$P<0·05$; ††$P<0·01$.
‡ For details of animals and procedures, see p. 1031.
ND, not determined.

animals, although no differences in plasma NEFA levels could be detected. Additional studies are required to establish the intrinsic mechanism for the decreased insulin release and enhanced insulin sensitivity for glucose utilization in the LL rats.

The decreased fat depots and altered lipoprotein profile observed in the LL male rats and the minor changes in females deserve some comment. The underfeeding condition during suckling clearly impairs long-term body growth in males, with a lesser effect in females. By analysing the weight of different tissues, it can be concluded that this difference in males mainly corresponds to fat depots, since both lumbar fat and epididymal fat pads weighed less in LL rats than in controls, in both absolute values and after correcting by body weight. Since plasma leptin levels have normally been associated with adipose tissue mass (Considine, 1997; Ahima & Flier, 2000), this difference in fat depots between male LL and control rats is further emphasized by the consistent lower plasma levels of their leptin. These reduced adipose tissue depots in adult LL males could be contributing to their enhanced insulin sensitivity, since it is known that removal of visceral fat prevents insulin resistance in rats (Gabriely *et al.* 2002). In fact, as the rats become older, a condition of insulin resistance develops, as is shown by the progressive increment of the AUC for insulin. In other words, as the animals become older, more insulin is required to maintain a normal glucose tolerance and this effect seems to be delayed in LL rats

Table 3. Body and tissue weights in 20-month-old rats suckled in control and large litter (LL) sizes‡
(Values are means with their standard errors for seven to twelve rats per group)

	Body wt (g)		Body size (cm)		Liver (g)		Liver (g/100 g body wt)		Kidney (g)		Kidney (g/100 g body wt)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Male												
Control	631·8	27·2	28·2	0·23	15·51	0·85	2·45	0·05	3·66	0·09	0·58	0·01
LL	485·4***	19·0	26·8**	0·38	11·23**	0·79	2·29	0·08	2·92***	0·16	0·60	0·02
Female												
Control	356·2†††	23·5	24·0†††	0·59	7·39†††	0·55	2·07†††	0·04	2·17†††	0·12	0·63	0·05
LL	301·8†††	15·7	23·4†††	0·23	6·49†††	0·30	2·16	0·06	1·72**†††	0·01	0·58	0·03
Significance of effect of:												
Gender	0·000		0·000		0·000		0·000		0·000		NS	
Litter size	0·000		0·02		0·002		NS		0·000		NS	
Gender × Litter size	NS		NS		0·035		NS		NS		NS	

Mean values were significantly different from those for the control rats: *$P<0·05$; **$P<0·01$; ***$P<0·001$.
Mean values were significantly different from those for the male rats: ††$P<0·01$; †††$P<0·001$.
‡ For details of animals and procedures, see p.1031.

Table 4. Tissue weights in 20-month-old rats suckled in control and large litter (LL) sizes‡
(Values are means with their standard errors for seven to twelve rats per group)

	Lumbar adipose tissue (g)		Lumbar adipose tissue (g/100 g body wt)		Periuterine adipose tissue (g)		Periuterine adipose tissue (g/100 g body wt)		Epididymal fat pads (g)		Epididymal fat pads (g/100 g body wt)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Male												
Control	17·56	3·08	2·65	0·37					11·78	1·44	1·81	0·18
LL	5·92**	0·93	1·20**	0·18					5·33***	0·86	1·07**	0·14
Female												
Control	7·08††	1·14	1·91	0·20	5·12	0·50	1·44	0·10				
LL	4·49	0·77	1·45	0·20	3·69	0·70	1·18	0·15				
Significance of effect of:												
Gender	0·016		NS									
Litter size	0·005		0·004		NS		NS		0·001		0·01	
Gender × Litter size	NS		NS									

Mean values were significantly different from those for the control rats: *$P<0·05$; **$P<0·01$; ***$P<0·001$.
Mean values were significantly different from those for the male rats: ††$P<0·01$; †††$P<0·001$.
‡ For details of animals and procedures, see p.1031.

Table 5. Plasma lipoprotein profile in 20-month-old rats suckled in control and large litter (LL) sizes‡
(Values are means with their standard errors for seven to twelve rats per group)

	TG (mg/l)		VLDL-TG (mg/l)		HDL-TG (mg/l)		Cholesterol (mg/l)		VLDL-cholesterol (mg/l)		HDL-cholesterol (mg/l)		LDL-cholesterol (mg/l)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Male														
Control	1538	234	1068	147	130	9	771	51	706	51	330	25	405	44
LL	752**	95	538*	92	156	109	581*	54	569	54	374	49	171**	3·7
Female														
Control	1075	145	742	124	175†	16	547††	35	555	35	284	25	207††	28
LL	1087	156	751	120	137	15	1052***†††	75	624	75	616**†	66	374*†	67
Significance of effect of:														
Gender	NS		NS		NS		0·035		NS		0·024		NS	
Litter size	NS		NS		NS		0·008		NS		0·000		NS	
Gender × Litter size	0·05		NS		0·02		0·000		NS		0·001		0·000	

Mean values were significantly different from those for the control rats: *$P<0·05$; **$P<0·01$; ***$P<0·001$.
Mean values were significantly different from those for the male rats: †$P<0·05$; ††$P<0·01$; †††$P<0·001$.
‡ For details of animals and procedures, see p.1031.
TG, triacylglycerol.

Table 6. Liver lipid component in 20-month-old rats suckled in control and large litter (LL) sizes‡
(Values are means with their standard errors for seven to twelve rats per group)

	TG (μmol/g)		Fatty acids (μmol/g)		Cholesterol (μmol/g)		Esterified cholesterol (μmol/g)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Male								
Control	21·01	2·84	1·18	0·16	5·99	0·52	1·45	0·17
LL	14·16*	1·49	1·06	0·17	6·36	0·61	1·53	0·12
Female								
Control	22·29	4·77	1·06	0·09	8·28†	0·93	1·33	0·10
LL	21·57	3·51	2·22**†	0·41	3·76***††	0·35	0·56***†††	0·08
Significance of effect of:								
Gender	NS		0·023		NS		0·001	
Litter size	NS		0·024		0·003		0·030	
Gender × Litter size	NS		0·006		0·001		0·009	

Mean values were significantly different from those for the control rats: *$P<0·05$; **$P<0·01$; ***$P<0·001$.
Mean values were significantly different from those for the male rats: †$P<0·05$; ††$P<0·01$; †††$P<0·001$.
‡ For details of animals and procedures, see p. 1031.
TG, triacylglycerol.

compared with control rats, and in females as compared with males in both groups. In fact, whereas the AUC for glucose was kept stable in male and female rats of both the LL and control groups, the AUC for insulin was lower in LL than in the control groups, and in females than in male rats, although the differences did not reach statistical significance at certain time points (Table 7).

The greatly impaired capacity to build up fat depots in male LL rats could also be responsible for the hypolipidaemia seen in these animals when 20 months old. Decreased fat depots could be responsible for the persistent decline in plasma TG as well as the low liver TG concentration seen in LL males. The low lipid substrate for VLDL synthesis would reduce liver VLDL-TG production, which may explain the low plasma levels in these animals. However, this reasoning cannot be used to understand the changes in the lipoprotein profile observed in female LL rats, where in addition to unchanged plasma VLDL levels, a clear hypercholesterolaemia corresponding to both increased HDL- and LDL-Ch levels was developed. In these animals, adipose tissue depots are less impaired

Table 7. Glucose tolerance tests at different ages in 20-month-old rats suckled in control and large litter (LL) sizes‡
(Values are means with their standard errors for seven to twelve rats per group)

Age (months)	4		10		16	
	Mean	SEM	Mean	SEM	Mean	SEM
AUC for glucose (mg/dl per min)						
Male						
Control	11 873[a]	268	10 765[b]	338	10 397[b]	219
LL	11 275[a]	289	10 306[a]	446	10 198[a]	759
Female						
Control	11 716[a]	439	9852[b]	261	10 749[ab]	651
LL	11 097[a]	593	10 747[a]	504	10 464[a]	630
Significance of effect of:						
Gender	NS		NS		NS	
Litter size	NS		NS		NS	
Litter size × Gender	NS		NS		NS	
AUC for insulin (pM/ml per min)						
Male						
Control	14 673[a]	2551	26 086[b]	3975	34 868[b]	3137
LL	8305[a*]	1128	15 439[ab*]	2407	24 120[b*]	3188
Female						
Control	9931[a]	130	12 461[a]††	2132	20 181[a]†	3795
LL	6296[a**]	658	12 613[a]	1778	9461[a*]††	918
Significance of effect of:						
Gender	NS		0·005		0·000	
Litter size	0·006		NS		0·001	
Litter size × Gender	NS		NS		NS	

AUC, area under the curve.
[a,b]Mean values within a row with unlike superscript letters were significantly different and indicate statistical comparison over the entire study period ($P<0·05$).
Mean values were significantly different from those for the control rats: *$P<0·05$; **$P<0·01$.
Mean values were significantly different from those for the male rats: †$P<0·05$; ††$P<0·01$.
‡ For details of animals and procedures, see p. 1031.

than those in males, allowing a normal production of VLDL, while liver Ch concentration is greatly reduced, probably as a consequence of enhanced liver production of HDL and/or decreased LDL uptake. A sexual dimorphism concerning those changes in lipoprotein liver production has been documented (Tessitore *et al.* 2000), and an inverse relationship between liver Ch concentration and plasma HDL levels has been reported previously in female rats (Munilla & Herrera, 1997). This suggests either an enhanced liver production of HDL, which would cause a depletion of liver Ch content or an enhanced bile Ch excretion in the presence of an enhanced reverse Ch metabolism. Present data do not permit us to decide between these two possibilities.

In summary, under-nutrition during suckling permanently decreases glucose-stimulated insulin secretion in male and female rats without affecting glucose tolerance. Whereas in males, it greatly impairs fat depot accumulation and reduces VLDL-TG in adults, it does not affect fat depot accumulation in females and has a hypercholesterolaemic effect, mainly corresponding to increments in plasma LDL- and HDL-Ch levels.

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