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Attenuation of obesity by early life food-restriction in genetically hyperphagic male OLETF rats: Peripheral mechanisms

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Abstract

The alarming increase in childhood, adolescent and adult obesity has exposed the need for understanding early factors affecting obesity and for treatments that may help prevent or moderate its development. In the present study, we used the OLETF rat model of early-onset hyperphagia induced obesity, which become obese as a result of the absence of CCK_1 receptors, to examine the influence of partial food restriction on peripheral adiposity-related parameters during and after *chronic* and *early* short term food restriction. Pair feeding (to the amount of food eaten by control, LETO rats) took place from weaning until postnatal day (PND) 45 (*early*) or from weaning until PND90 (*chronic*). We examined fat pad weight (brown, retroperitoneal, inguinal & epididymal); inguinal adipocyte size and number; and plasma leptin, oxytocin & creatinine levels. We also examined body weight, feeding efficiency and spontaneous intake after release from food-restriction. The results showed that *chronic* food restriction produced significant reductions in adiposity parameters, hormones and body weight, while *early* food restriction successfully reduced long term body weight, intake and adiposity, without affecting plasma measurements. *Early* (and *chronic)* dieting produced promising long term effects that may imply the reorganization of both peripheral and central mechanisms that determine energy balance and further support the theory suggesting that early interventions may effectively moderate obesity, even in the presence of a genetic tendency.

Keywords

food restriction; obesity; animal models; early-onset obesity

Introduction

Obesity is a leading preventable cause of death worldwide, with growing prevalence among children and adults, and is currently viewed as one of the most serious public health problems

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of the 21st century. Overweight subjects face the challenge of losing weight and then maintaining the results in the long term, without relapsing to the obese state, a goal that has proven over time as being extremely difficult to achieve. Once obesity is established in adulthood, interventions such as diets, exercise and medication provide only temporary solutions, and subjects usually regain most of the weight lost in a relatively short period of time (Franz et al., 2007; Wadden, 1993).

Research performed on animals has led to the theory that interventions during critical (early) periods during development may lead to a reorganization of both central and peripheral mechanisms involved in energy balance, maybe even resetting genetic predispositions (Aggoun, 2007; Barker, 1998, 2002; Miller & Silverstein, 2007; Plagemann, 2006; Taylor & Poston, 2007). Still, only lately have studies started examining the early developmental stages and their importance determining later overweight and adiposity (Bouret, 2009; Patterson et al., 2008; Zhang et al., 2007).

The Otsuka Long Evans Tokushima Fatty (OLETF) rats are a model of non-insulin dependent diabetes mellitus (NIDDM) (Kawano et al., 1994; Kawano et al., 1992) and early-onset hyperphagia- induced obesity (Moran & Bi, 2006; Schroeder et al., 2007b; Schroeder et al., 2009a, b) that has a congenital defect in the expression of the cholecystokinin-1 (CCK_1) receptor gene (Nakamura et al., 1998). CCK is a brain-gut peptide that acts as a peripheral satiety molecule (Smith, 2006; Weller, 2006) and elicits the earlier appearance of the behavioral satiety sequence (Gibbs et al., 1973), limiting the size of the meals. The absence of this signal causes chronic hyperphagia that eventually induces OLETF males and females to become obese and hyperleptinemic (Moran & Bi, 2006; Schroeder et al., 2009b). OLETF rats are overweight and hyperphagic from birth (Blumberg et al., 2006; Schroeder et al., 2006, 2007a, b) and develop increased adiposity and adipocyte hypertrophy from postnatal week 1 (Schroeder et al., 2009a) compared to LETO (Long Evans Tokushima Otsuka) controls. Explicit obesity in this strain emerges later in life (around PND40–45 in the males) (Schroeder et al., 2009b) and only then several other systems appear to eventually become dysregulated, worsening their already pre-obese phenotype. Such is the case for leptin (Niimi et al., 1999), oxytocin (Hashimoto et al., 2005; Schroeder et al., 2009b; Zagoory-Sharon et al., 2008) and dopamine (Anderzhanova et al., 2007; Feifel et al., 2003; Hajnal et al., 2007, 2008).

Food restriction or “pair feeding” (PF) to the levels of food eaten by the lean, control strain, is a strategy frequently used in genetic animal models of obesity. PF was performed on ob/ob or db/db mice (Coleman, 1978) and obese Zucker rats (Cleary et al., 1980, 1987; Johnson et al., 1997). These animals maintained their degree of relative adiposity even when subjected to early-life marked food restriction at a level similar to or lower than the food consumed by their lean counterparts. This response to food restriction, usually distinguishes genetic from non-genetic (e.g., Wistar and Sprague Dawley rats) rodent models. For non-genetic rodent models, underfeeding early in life leads to reduced rates of body weight, body fat, limits age-related increases in fat cell size and number and reduces leptin levels (Hausman et al., 2003). PF has been performed on male obese OLETF rats in order to examine the potential role of hypothalamic pathways in their hyperphagia and obesity (Bi et al., 2001) and the contribution of the obese phenotype to NIDDM development in males of this strain (Man et al., 2000, Park et al., 2005). In those studies, PF normalized NPY and POMC expression in the arcuate nucleus, implying improved sensitivity to peripheral leptin, and improved whole body glucose disposal and insulin resistance (Man et al., 2000; Park et al., 2005). Food restriction even prevented NIDDM when started at 5 weeks of age (Okauchi et al., 1995) and reduced the cardiovascular risk factors provoked by diabetes mellitus in adult OLETF males (Minamiyama et al., 2007). In contrast to the above mentioned genetic models of obesity, OLETF males under PF showed normalized body weight, body fat, leptin and insulin (Bi et al., 2001). The OLETFs response to PF is therefore in accordance with non-genetic rat models, probably because obesity in this

strain is secondary to hyperphagia, showing them to be a very useful model for the study and prevention of overeating induced obesity.

In the present study, we aimed to analyze: 1) The effect of early (weaning-onset) chronic food restriction on adipose tissue growth and cellularity and on circulating levels of leptin, oxytocin and creatinine in the developing animals; and 2) the long term effect of early short term PF on body weight, intake, adiposity, cellularity and selected plasma measurements after resumption of ad libitum feeding by previously food-restricted obese rats.

Lack of perseverance (in dieting or exercising) is usually the factor representing the biggest challenge for patients intending to either loose weight or maintain their body weight after weight loss. In line with the Barker hypothesis, the main purpose of this study was to examine the early post-weaning period (childhood for humans) as a potential target for treatment (or partial prevention) that could lead to a long lasting reduction in body adiposity/intake/body weight threshold, reducing obesity levels and its related health risks in the long term.

Methods

Subjects

OLETF and LETO males were raised in our colony at the Developmental Psychobiology Laboratory in Bar-Ilan University, Ramat-Gan, Israel. The original rats were received as a generous gift from the Tokushima Research Institute, Japan. Newborn litters were culled to 10 pups (minimum 7), with sex distribution kept as equal as possible in each litter. OLETF and LETO offspring were housed with their dams until weaning. During the period of food restriction, males were housed individually, but during the re-feeding periods they were housed in pairs in order to reduce the isolation stress. Polycarbonate cages (18.5 cm height × 26.5 cm width × 43 cm length) were used, with stainless steel wire lids and wood shavings as bedding material. Food (Koffolk 19510, 4% fat) and water were freely available (except for during the PF manipulation). The animals were on a 14:10 hr light: dark cycle, with lights on at 05:00. Room temperature was maintained at 22+/−2 °C.

The research protocol was approved by the Institutional Animal Care and Use Committee, and it adhered to the guidelines of the American Psychological Association and the Society for Neuroscience.

Experimental procedure

Experiment 1: Chronic pair feeding—At the time of weaning, males were placed in a pair-fed regimen, where they received the daily average amount of food consumed by same aged LETO controls. Days of sacrifice included PND22, 38, 65 and 90 to examine the influence of chronic diet on developing males.

Experiment 2: Early short-term pair feeding—The pair feeding regimen started at weaning and was continued until PND45. From that point, rats were given renewed access to *ad libitum* food until the day of sacrifice, on PND90.

In addition, LETO and normal-fed OLETF males were maintained with *ad libitum* access to standard chow; they were reared in pairs and served as controls. In addition, a few males were reared alone and their intake and body weight was assessed. No significant differences in body weight, intake or total fat were found in response to individual vs. paired housing in control rats of either strain, so their data was pooled.

Both experiments were performed in parallel; with one control group with *ad lib* access of each strain. Six to eight animals were used per group and sacrifice age.

Body weight and intake

Rats were weighed every fifth day from weaning on PND 22–23 until PND 90 or 120 depending on the diet time-window. Intake was assessed daily from pairs of rats starting at the time of weaning (PND 22). Feeding efficiency was calculated (5 day -body weight gain/5 day-intake in grams).

Tissue collection

Experiment 1—Four sacrifice time-points were chosen throughout development in order to examine the influence of chronic food restriction on developmental adiposity in the OLETF strain. PND22–23 represented the starting point of the study. PND38 was chosen as the developmental period preceding the emergence of obesity in OLETF males. PND 65 and 90 represented early and mid-adulthood and are critical points in obesity development in both sexes (Schroeder et al., 2009). Control LETO and OLETF animals were also sacrificed at each of the time-points.

Experiment 2—The animals in the early short term manipulation were sacrificed on PND90. On the day of sacrifice, rats were weighed and sacrificed between 11:00 AM and 2:00 PM (in both experiments). Interscapular brown adipose tissue (BAT), Retroperitoneal (Retro), inguinal (IAT) and epididymal adipose tissues were collected from decapitated animals, weighed and a sample of the inguinal fat pad was immediately frozen on dry ice. Trunk blood for leptin, creatinine and oxytocin analyses was collected in chilled heparinized vacutainer tubes coated with EDTA. Samples were preserved at −80°C until analyzed.

Plasma measurements

Plasma leptin, oxytocin and creatinine levels were assessed using commercial ELISA kits (R&D Systems, Minneapolis, USA for the first two and Cayman, Michigan, USA, respectively) according to the manufacturers' instructions. In order to assess the effects of food restriction on muscle mass loss, plasma creatinine was analyzed and used as an indirect estimation of muscle mass. In following with our recent findings showing that plasma oxytocin levels were very high and in correlation with leptin and the amount of white fat in OLETF males and females (Schroeder et al., 2009b), we decided to test this correlation by examining the changes in this hormone when obesity development is avoided.

For leptin, intra-assay precision was 3.8%, inter-assay precision 5.7%, with an average of 96% recovery. For creatinine, intra-assay precision was 2.7%, inter-assay precision 3%, with an average of 95% recovery. For oxytocin, intra-assay precision was 12%, inter-assay precision 5%, with an average of 93% recovery.

Histology

Samples of the inguinal white adipose tissue (IAT) were used to characterize adipocyte cell size. Tissues were sectioned to 8 micrometers by a Cryostat (Leyca) at −35 °C and mounted on glass slides. Digital photographs were taken using the ACT1 program, at × 200 magnification. For each inguinal fat pad examined, 10–20 pictures were taken from 3 different zones of the sample, with at least 100 micrometers distance from each other. Adipocyte size parameters were derived from 3 to 6 representative cells from each picture, depending on the size of the cell, using the public domain National Institutes of Health Scion image program. For each animal, at least 60 cells were analyzed. Representative cells chosen presented a smooth and clear membrane, with no granulation around. A similar methodological approach was described elsewhere (Schroeder et al., 2009a, 2009b; Zagoory-Sharon et al., 2008). The estimated number of cells per fat pad was calculated using the average diameter, a density

conversion factor (0.915 g/cc), and the mass of the fat pads, as previously described (Ashwell et al., 1976; MacLean et al., 2006).

Statistical approach

Group differences (LETO, OLETF [free-feeding], *chronic and early* PF) in BW and FE were analyzed by repeated measures ANOVA comparing the 4 groups (independent variable) over the repeated days of measurement. This was followed up by one-way ANOVAs comparing the 4 groups at each of the ages, with post-hoc Duncan's test ($p<0.05$) for pairwise comparisons. In Experiment 1, group differences in adiposity and plasma measures were similarly analyzed by one-way ANOVAs comparing the 3 groups (LETO, OLETF and *chronic* PF) at each of the ages, with post-hoc Duncan's tests. In Experiment 2, group differences in intake were examined on the LETO, OLETF and *early* PF groups from PND45 and on by a similar ANOVA. Group differences in adiposity and plasma measures on PND90 were analyzed by one-way ANOVAs comparing 3 groups (LETO, OLETF and *early* PF), with post-hoc Duncan's tests. *Chronic* PF data on PND90 were included in the figures for comparison purposes. Differences between ad lib fed OLETF and LETO controls were not the focus of this paper and were not highlighted in the results and figures, because they were published in a previous study (Schroeder et al., 2009b).

Results

Body weight, food intake and feeding efficiency

Both manipulations affected body weight over time ($F(42,30)= 22.07$, $p<0.001$ for the interaction effect; ANOVAs starting on PND30: $F>18.81$, all $p<0.001$; Fig. 1A). Duncan's tests revealed that the early short-term diet reduced BW, compared to free-feeding OLETF rats, beyond the food-restriction period, throughout the remainder of the study (Fig. 1A). Compared to these controls, *early* diet restriction affected food intake over time ($F(24,16) =6752.53$, $p<0.001$) for the interaction effect; ANOVAs at all ages $F> 23.28$, all $p<0.001$). Duncan's tests revealed that release from the early short-term diet was followed by one day of relative hyperphagia, after which the rats maintained spontaneous eating levels that were consistently below those of control OLETF rats (Fig. 1B). The pattern of FE development was different in the different groups ($F(26,8)=10.57$, $p<0.001$ for the age × group interaction; ANOVAs at all ages but 25, 35, 80 & 90: $F>3.84$, $p<0.05$), with both PF groups presenting frequent fluctuations in their feeding efficiency (Fig. 1C).

Experiment 1: Chronic pair feeding throughout development

The chronic PF normalized the weight of the white fat pads (inguinal, epididymal, & retroperitoneal), expressed as percent of the rat's BW) to that of the LETO controls on PND38, 60 and 90 in the first two tissues, on PND38 & 90 in the retroperitoneal tissue and on PND90 in the brown tissue; as shown in Fig. 2. On PND38, the manipulation even further reduced all white fat levels, significantly below LETO levels (all $Fs>4.53$, all $p<0.05$, Duncan's tests, $p<0.05$). Raw weight of the fat pads is presented in table 1 (significance according to Duncan's tests, $p<0.05$). As can be seen in Fig. 3A, chronic PF normalized adipocyte size on PND65 & 90, compared to OLETF controls ($Fs>8.17$, all $p<0.01$, Duncan's tests; $p<0.05$) without affecting the estimated adipocyte number (Fig. 3B) but significantly increasing the relative adipocyte number from PND65 (both $Fs>0.05$) (Fig. 3C).

Chronic PF decreased plasma leptin levels compared to OLETF controls, at all 3 ages (all $Fs>6.04$, all $p<0.05$, Duncan's tests; Fig. 4A). While group differences were not found on PND38 in plasma oxytocin levels, PF significantly normalized oxytocin to LETO levels on PND65 & 90 (both $F>5.39$, $p<0.05$; Fig. 4B).

Experiment 2: Short term pair feeding

Early diet-restriction had significant effects on adiposity, compared to OLETF controls ($p<0.001$ for inguinal (Fig 5A), $p<0.001$, retroperitoneal (Fig 5B, $p<0.001$) epididymal (Fig 5C and $p<0.05$) and brown fat (Fig 5D) pads. Inguinal adipocyte size was also significantly reduced ($F(2,15) =31.28$, $p<0.001$ (Fig 6A), without affecting the estimated adipocyte number (Fig 6B). Interestingly, early PF only tended to increase the relative number of adipocytes when normalized to body weight ($p=0.063$; Fig. 6C). Plasma leptin, oxytocin and creatinine levels were not significantly affected in adulthood by *early* diet-restriction: PF levels on these measures did not differ from those of OLETF controls at the end of the follow up (Duncan's test, $p<0.05$; Fig. 7).

Discussion

The prevention of weight regain has emerged as the most significant obstacle in combating the obesity epidemic. Extensive research has shown that, when performed late in life, food restriction usually provides only a transitory solution and subjects quickly return to their "baseline" body weight soon after termination of the diet regimen. Such is the case for Sprague-Dawley DIO rats (Levin & Dunn-Meynell, 2000) and obesity-prone and obesity-resistant Wistar rats (MacLean et al., 2004). The study of adult animals has led to results showing that the energy gap increases with time in the weight reduced state (MacLean et al., 2004), dispelling the hopes that the metabolic drive to regain weight may eventually dissipate if intake could be restricted long enough for the homeostatic system to readjust (MacLean et al., 2006).

The importance and involvement of the peripheral tissues in the relapse-after-diet phenomenon has lately become the focus of many studies (Bays et al., 2006, 2008; Gustafson et al., 2009; MacLean et al., 2006).

When chronically food restricted, OLETF males showed a marked normalization in plasma leptin, oxytocin and adiposity that was related to a reduction in fat cell size. Moreover, *chronic* pair feeding even increased their relative fat cell number, normalizing them to LETO controls.

Pathogenic adipose tissue (malfunctioning of adipocytes) is associated with many of the common metabolic diseases, like type II diabetes, hypertension and dyslipidemia, all of them present in the obese OLETF male (reviewed by Moran, 2008). If adipogenesis is impaired during positive caloric balance, then existing adipocytes must undergo hypertrophy in order to store the excessive energy. Adipocyte hypertrophy may then be a result of the failure of adipocytes to adequately proliferate (Bays et al., 2008). Since the enlargement of adipocytes is associated with substantial changes in metabolic functions (such as leptin- and insulin-resistance), it has been hypothesized that such alterations may contribute to the health risks of obesity. Alterations in adipocyte function have been reported in adult OLETF males in regard to their diabetes, and interestingly, caloric restriction improved their situation (Park et al., 2005). Fat cells release a variety of adipokines and many other biologically active molecules (Ailhaud, 2006) which may be involved in the development of a chronic low-grade inflammatory state that may, in turn, underlie the pathogenesis of the metabolic and cardiovascular complications of obesity. Large fat cells secrete higher amounts of adipokines such as leptin, IL-6, IL-8, TNF-alpha and adiponectin (among others), compared to small cells (Skurk et al., 2007). In contrast to the increase in adipose cell size in obesity, obese subjects tend to have fewer of adipocytes (Gustafson et al., 2009; Isakson et al., 2009) and the amount of pre-adipocytes that can undergo differentiation is reduced in hypertrophic obesity (Isakson et al., 2009; Permana et al., 2004). Moreover, the capacity of pre-adipocytes to differentiate to adipose cells appears to be negatively correlated with both BMI and adipocyte cell size (Isakson et al., 2009).

The adipocyte profile we found in free-feeding OLETF males is similar to the one described above. OLETF males develop adipocyte hypertrophy early in life, with a significant reduction in the relative amount of cell number (Schroeder, et al., 2009a, b). While *early* pair feeding successfully reduced cell size in the long term, it was the *chronic* manipulation that in addition to cell size reduction also normalized the relative number of adipocytes to those of LETO controls. This combination of changes may imply an improvement in fat cell functioning that may be related to the improvement in diabetes reported by Park et al (2005).

Food restriction is associated with suppressed thermogenesis in brown adipose tissue and increased metabolic efficiency. Relative BAT weight (Rodriguez-Cuenca et al., 2002) and UCP1 content are reduced in animals under food restriction, probably indicating a decrease in BAT thermogenic activity (Rothwell & Stock, 1982; Valle et al., 2005). Thus, the reduced energy expenditure which occurs during food restriction may be partly related to a lower activity of brown adipose tissue (Rothwell & Stock, 1982). In adult pre-diabetic OLETF males, UCP1 (regulator of thermogenesis and body composition) and UCP3 are spontaneously reduced (Ryu et al., 2003). This may cause diminished energy dissipation that could contribute to the development of obesity. Unfortunately, under chronic food restriction UCP1 and UCP3 are reduced even further in OLETF males (Ryu et al., 2003). Similarly, in the present study, we found a significant reduction in BAT mass in *chronic* pair feeding. In addition, toward the end of the *chronic* pair feeding period, the feeding efficiency decreased significantly, showing that even if there is reduced energy dissipation by the BAT, OLETF males are not able to maintain their trajectory of weight gain (see Fig 1A). This profile was not observed in the *early* PF group. Thus, chronic pair feeding does not correct the OLETF pathology in this regard; it even appears to worsen the situation. In the *early* pair fed animals the amount of BAT was not altered on PND90 compared to free-feeding OLETF rats. Overall, both of our manipulations seem to have had little (or even negative) effect on OLETF BAT mass (and hypothetically also function).

Oxytocin neurons in the PVN play a role in coordinating feeding termination (Blevins et al., 2003; Olson et al., 1991a, b; Verbalis et al., 1993) by acting as targets for factors which induce anorexigenic behavior such as CCK (Olson et al., 1992) and leptin (Hakansson et al., 1998; Ur et al., 2002). Central and peripheral concentrations of oxytocin are related and both are elevated in pathological states of energy balance such as obesity (Smith, 2006; Stock et al., 1989). One study further reported that oxytocin levels decreased significantly following gastric banding, a procedure that induced weight loss in the patients (Stock et al., 1989). Accordingly, *chronic* pair feeding in our model normalized plasma oxytocin (and leptin) to LETO controls.

The findings related to body weight and adipose tissue appear very promising, and while only the *chronic* manipulation achieved a normalized (to LETO) profile, *early* pair feeding produced significant long lasting effects on obesity levels that may imply perdurable improvements in the animals' health. It was somewhat disappointing that the long term improvements just mentioned were not reflected by leptin and oxytocin levels at Day 90. The most promising result of this short-term manipulation was the reduction in voluntary intake observed in this group, which may suggest reorganization of central pathways implicated in energy regulation. If this is the case, this long term reduction in intake may lead to future reductions in adiposity and leptin and oxytocin levels that may improve long term health.

The present study provides further insights into the peripheral mechanisms underlying adiposity related adaptations in response to *chronic* and *early* pair feeding in OLETF males. While *chronic* pair feeding leads to an effective normalization of almost all the examined obesity parameters, such a prolonged food restriction is unlikely to be achieved in human subjects. Moreover, the reduction in plasma creatinine (though not significant) suggests a strong reduction in lean body mass that could be difficult to recover. On the other hand, the

early manipulation appears as a promising option for long term obesity reduction. A more moderate food restriction for a longer period of time or even the combination of it with exercise (which by itself successfully moderates long term obesity (Bi et al., 2005) during childhood may provide long term results that cannot be achieved once the central and peripheral systems related to energy balance are mature and can hardly be modified.

Understanding the peripheral mechanisms by which adiposity is reconstituted after weight loss should be of high priority since it may lead to new ways of treating obesity and its associated metabolic complications. By extensively characterizing this model of weight regain, we may be able to use it to identify nutritional, behavioral, and pharmacological strategies that could counter the propensity to regain weight and would hopefully facilitate long-term weight reduction in obese and overweight subjects.

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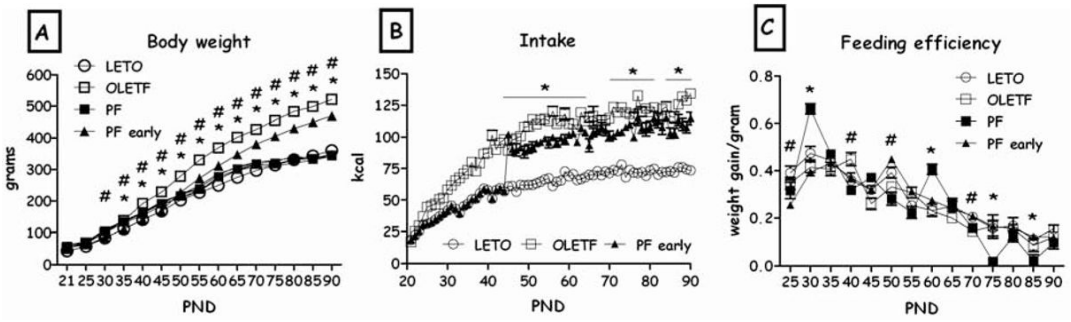

Fig. 1.

OLETF (free-feeding controls), LETO (free-feeding controls), *chronic* and *early* pair-feeding OLETF males' body weight in grams **(A),** intake in kcal **(B)** and feeding efficiency (weight gain/grams eaten) **(C)** from PND 22 to 90. In the intake figure, the *chronic* PF is not included since daily intake was equal to the amounts consumed by LETO controls. Data are presented as means and SEM. *$p<0.05$ for significant differences between *chronic* pair-feeding and OLETF controls and #$p<0.05$ for significant differences between *early* pair-feeding and OLETF controls. N= 6–8 per group.

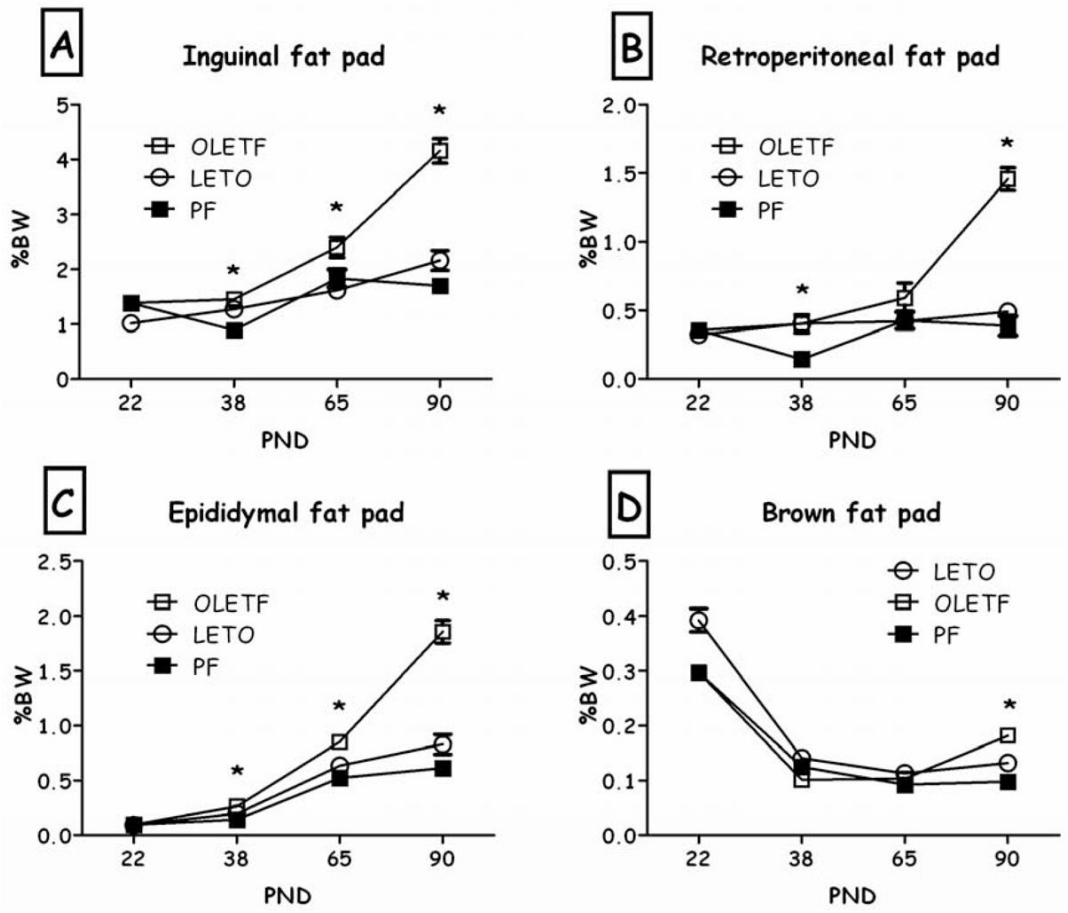

Fig. 2.

Percentage weight of the different fat pads of OLETF, LETO and *chronic* pair-feeding OLETF males on PND 22, 38, 65 & 90 (expressed as percent of BW). Inguinal fat pad **(A)**, retroperitoneal white fat (**B**), epididymal white fat (**C)** and brown fat (**D**). Data are presented as means and SEM. *p<0.05 for significant differences between *chronic* pair-feeding and OLETF controls. N= 6–8 per group.

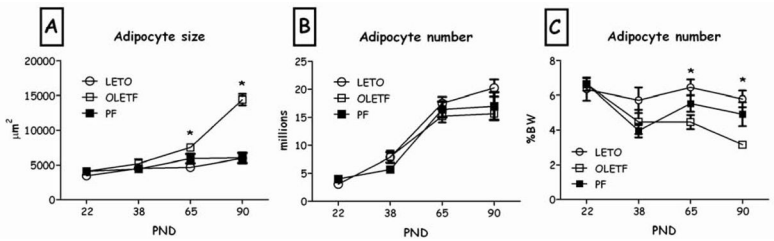

Fig. 3.

Adipocyte size **(A)**, estimated number of adipocytes **(B)** and relative adipocyte number (normalized to BW) **(C)** of OLETF, LETO and *chronic* PF OLETF males on PND22, 38, 65 & 90. Data are presented as means and SEM. *p<0.05 for significant differences between *chronic* pair-feeding and OLETF controls. N= 4–6 per group.

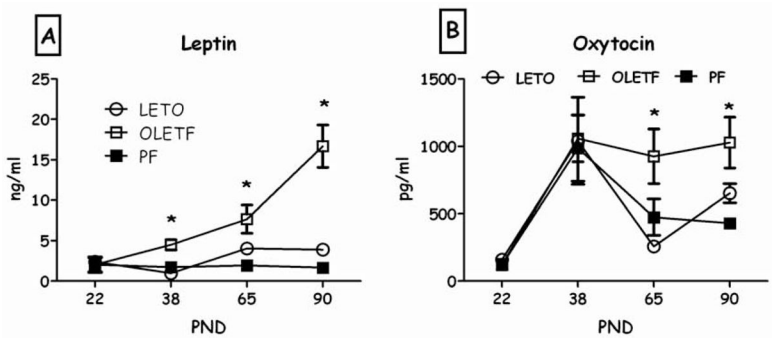

Fig. 4.

Plasma leptin **(A)** and oxytocin **(B)** levels of OLETF, LETO and *chronic* pair-feeding OLETF males on PND22, 38, 65 & 90. Data are presented as means and SEM. *p<0.05 for significant differences between *chronic* pair-feeding and OLETF controls. N= 4–6 per group.

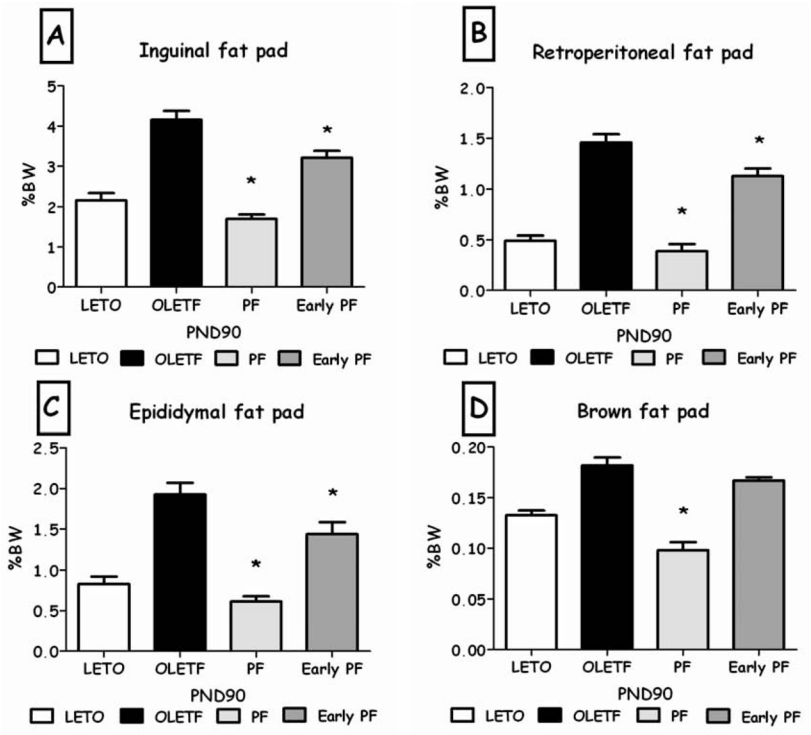

Fig. 5.

Percentage weight of the different fat pads of OLETF, LETO, *chronic* and *early* pair-feeding OLETF males on PND90 (expressed as percent of BW). Inguinal fat pad **(A)**, retroperitoneal white fat (**B**), epididymal white fat (**C)** and brown fat (**D**). Data are presented as means and SEM. *p<0.05 for significant differences between the pair-feeding manipulations and OLETF controls. N= 6–8 per group.

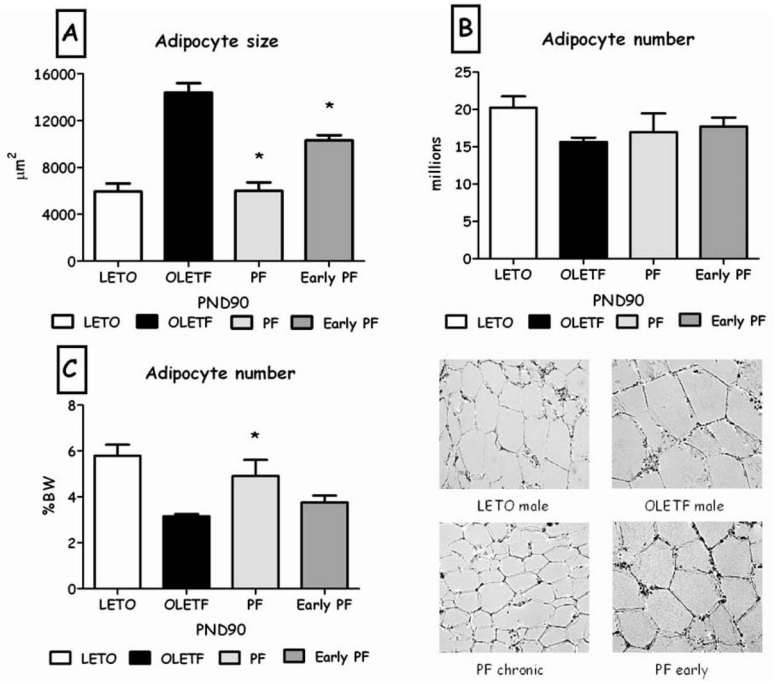

Fig. 6.

Adipocyte size **(A)**, estimated number of adipocytes **(B)** and estimated number of adipocytes expressed as percent of BW of OLETF, LETO *chronic* and *early* PF OLETF males on PND90. Data are presented as means and SEM. *$p<0.05$ for significant differences between the pair-feeding manipulations and OLETF controls. N= 4–6 per group. The LETO, OLETF and PF data are the same as presented in Figure 3 and are included here for comparison.

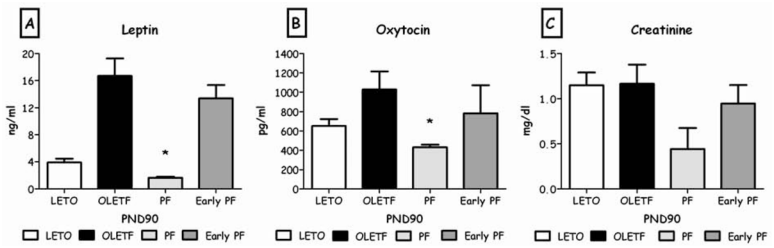

Fig. 7.

Plasma leptin **(A),** Oxytocin **(B)** and Creatinine **(C)** levels OLETF, LETO, *chronic* and *early* pair-feeding OLETF males on PND90. Data are presented as means and SEM. *$p<0.05$ for significant differences between the pair-feeding manipulations and OLETF controls. N= 4–6 per group.

Table 1

Fat pad raw weight (in grams).

		LETO	OLETF	PF	PF early
PND23	**BAT**	0.22±0.03	0.17±0.00	0.17±0.00	0.17±0.00
	Retro	0.15±0.02	0.21±0.02	0.21±0.02	0.21±0.02
	Ingual	0.48±0.02	0.83±0.04	0.83±0.04	0.83±0.04
	Epy	0.04±0.00	0.06±0.01	0.06±0.01	0.06±0.01
PND38	**BAT**	0.18±0.01	0.19±0.01	**0.18±0.01**	
	Retro	0.54±0.07	0.75±0.11	**0.21±0.02***	
	Ingual	1.68±0.11	2.69±0.23	**1.28±0.08***	
	Epy	0.27±0.03	0.48±0.01	**0.21±0.01***	
PND65	**BAT**	0.31±0.01	0.37±0.03	**0.26±0.01***	
	Retro	1.18±0.14	2.13±0.41	**1.26±0.19***	
	Ingual	4.47±0.13	8.55±0.77	**5.38±0.50***	
	Epy	1.77±0.08	3.04±0.22	**1.55±0.15***	
PND90	**BAT**	0.48±0.03	0.91±0.03	**0.33±0.03***	**0.79±0.02***
	Retro	0.91±0.21	7.6±0.66	**1.34±0.24***	**5.33±0.36***
	Ingual	8.42±0.76	21.6±1.77	**5.85±0.37***	**15.11±0.93***
	Epy	3.29±0.39	9.56±0.81	**2.13±0.22***	**6.80±0.79***

*Significantly different from OLETF control group ($p<0.05$).