Towards an animal model of childhood-onset obesity:
Follow-up of OLETF rats during pregnancy and lactation

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Running head: OLETF obesity in pregnancy & lactation

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Abstract

The OLETF rat model of obesity (a spontaneous CCK$_1$ receptor knockout) has been extensively studied as model of hyperphagia-induced obesity. In previous studies, young OLETF rats presented abnormal eating patterns (compared to LETO controls) in a variety of independent ingestion and nursing tests during the suckling period. The aim of the present study was to characterize the early emergence of abnormal adiposity in the pups. Moreover, because both the dams and the pups present the genetic mutation, a close follow-up of the dams' body weight and intake during pregnancy and lactation was performed, in order to examine the circumstances that contribute to build up the pups' early adiposity. Compared to controls, OLETF pups presented higher fat levels, larger adipocytes and increased waist circumference as early as postnatal day 7 and this profile persisted to the age of weaning. While LETO dams gained weight throughout pregnancy and lactation, OLETF dams were obese and hyperphagic during pregnancy but lost weight during lactation, probably as a result of rearing hyperphagic pups. Current and previous results suggest a possible influence of the dams' obesity during gestation and a high investment in nursing time during lactation on the pups' obesity levels during childhood. This, combined with the innate hyperphagia repeatedly observed in the pups at these early ages, makes the OLETF strain a useful tool in the research of childhood-onset obesity.

Keywords: overeating, animal models, gestation, feeding.
Introduction

Obesity is a growing public health concern that has reached epidemic proportions in Western Societies in the last few decades (27). Although much research has already been conducted on obesity using animal models, investigations have usually focused on adult males, leaving a gap regarding childhood and female obesity. In many cases, early symptoms can be detected during "pre-obese" stages, where interventions could be more successful in reaching perdurable outcomes. Given the permanent upward resetting of body weight set point that takes place when genetically predisposed individuals become obese, the identification of the early factors that can help understand and prevent obesity might be the key to control the spread of the obesity epidemic (1, 5, 6, 33, 36).

Maternal obesity during pregnancy and lactation have been found to influence the offspring in the long term, predisposing them to develop larger fat pads and higher leptin and glucose levels later in life, especially in the presence of a genetic tendency (23, 30). Maternal obesity can also predispose offspring to obesity for generations to come (15, 47). In a study on mice, females fed a palatable obesogenic diet throughout pregnancy and lactation produced obese offspring presenting hyperphagia during adolescence and adulthood, with reduced locomotor activity and increased adiposity. Abdominal obesity in this model was associated with adipocyte hypertrophy (38). Exposure to a high-energy diet specifically during pregnancy (and not lactation) has been found to cause hyper-adiposity and reduced glucose tolerance in the neonatal/weanling offspring (14). Another study suggested that maternal obesity at conception leads to fetal programming of the offspring, which results in obesity later in life (44). In a further study, rat dams provided with a "cafeteria diet" (a variety of high fat/ palatable food) from the first day of pregnancy exhibited
hyperphagia throughout pregnancy and lactation, compared to chow-fed controls. Despite feeding on a cafeteria diet, these dams showed no body mass increase during lactation. This perhaps suggests a higher energy investment in milk production, which might have also been richer in energy and fat. Rats born to mothers fed a cafeteria diet developed an exacerbated preference for fat, sugar and salty foods and also exhibited increased body weight and BMI compared to other offspring. This study shows that a maternal high fat diet during pregnancy and lactation may be an important contributing factor in the development of obesity (7). Altogether, the results of these studies expose some consequences of the mothers' overeating (and probably of a high body fat percentage) at the time of fetal development and lactation on their offspring's short and long term obesity development.

The Otsuka Long Evans Tokushima Fatty (OLETF) rat model lacks the CCK₁ receptor due to a spontaneous genetic mutation, and represents a broadly established model of non-insulin dependent diabetes mellitus (NIDDM) (28, 29) and hyperphagia induced obesity (34). CCK is a brain-gut peptide which acts as a peripheral satiety molecule, through CCK receptors of type 1, as proved by extensive research with exogenous CCK, specific CCK₁ receptor antagonists and with the OLETF CCK₁ knockout model of obesity (for review see (45)). Adult OLETF males are hyperphagic, obese, hyperleptinemic, completely resistant to CCK administration, hyperglycemic, develop NIDDM, and present up-regulation of NPY mRNA in the DMH (10, 34, 35). OLETF females typically do not develop diabetes (50).

We have recently dedicated much effort into understanding the behavioral aspects of the "pre-obese" stages in the OLETF strain, and demonstrated that OLETF rats are heavier from birth
and show hyperphagic characteristics in independent ingestion tests as early as postnatal day (PND) 2 (12). OLETF pups also consume more milk during nursing bouts whether they are suckling from an OLETF or a LETO (Long Evans Tokushima Otsuka) control dam (41) and also initiate more nursing bouts, especially during the 3rd postnatal week (40). Overall, we concluded that an abnormal intake profile is observed very early during development, showing that obesity in this strain develops following lifelong abnormalities in eating behavior.

In addition to these differences between the pups, there is a disparity in the behavior of OLETF and LETO dams that may contribute to the difference in the rate of weight gain of the offspring (39, 40). Beyond the increased nursing time found in the OLETF strain, we have recently reported a detailed physiological characterization of the changes dams of both strains undergo throughout lactation (49). OLETF females appear to gradually lose weight and body fat after delivery, an outcome that hypothetically might be a consequence of larger milk production to feed hyperphagic pups. This contrasted with the pattern of weight gain over lactation that was apparent in control, LETO dams. That study, however, was cross-sectional, so our conclusions result from group comparisons rather than from individual follow-up data. Furthermore, that study did not include pregnant rats, starting the follow-up post-partum. Therefore, in the present study, it was of special interest to closely follow up the dams' changes in body weight and intake throughout pregnancy and lactation in order to identify further contributing factor to the overweight of the pups at birth and during the suckling period.

In the present study, we aimed: 1) To characterize the early adiposity-related development in the offspring from birth until weaning. Examination of this potentially critical time for obesity development when fat stores, body weight and intake change dramatically, may help to identify
possible markers that could successfully predict later obesity and provide time windows where interventions could be more successful in the treatment of long term obesity (36) (see "Barker hypothesis"; 5, 6); 2) To further explore the dams' contribution to the early development of obesity in the OLETF strain, by characterizing their intake and body weight during pregnancy and lactation.

**Materials and Methods**

**Subjects:**

Twenty seven nulli- and primiparous OLETF and LETO dams and their offspring were raised in the SPF facility of the Gonda Brain Research Center at Bar-Ilan University, Ramat-Gan, Israel. The original animals were received as a generous gift from the Tokushima Research Institute, Japan. Females were mated with males from their own strain. For the pregnancy follow-up, adult females were mated with males for only 1 night during the pro-estrous phase of the estrous cycle (determined by vaginal smears). If sperm was identified by a further vaginal smear the morning after, it was considered as gestation day 0. Pregnant females were housed in pairs until 3-4 days before parturition to avoid isolation stress. After parturition, OLETF and LETO dams and their offspring were housed together until weaning. Controls for the dams were group housed, not mated, and followed up at comparable ages. Clean polycarbonate cages (18.5 cm height x 26.5 cm width x 43 cm length) were used, with stainless steel wire lids and wood shavings as bedding material. Food (Harlan 2018SC+F (6% fat)) and water were freely available. The animals were on a 12:12 hr light: dark cycle, with lights on at 07:00. Room temperature was maintained at 22+/−2 °C. Newborn litters were culled on PND1, between 11:00 AM and 2:00 PM, to 10 pups (minimum 7), with sex distribution kept as equal as possible in each litter.
The research protocol was approved by the Institutional Animal Care and Use Committee, and it adhered to the guidelines of the APS’s Guiding Principles in the Care and Use of Animals and of the Society for Neuroscience.

**Body weight (BW) and intake**

Dams were weighed every fifth day from gestation day 0 (GD 0) until GD 20 and then from PND 1 until the time of weaning and intake was measured daily. Pups were weighed every fifth day from PND 1 until weaning (PND 22-23). Body mass index (BMI = body weight in grams / (body length in cm²)) and waist circumference were also examined as further obesity parameters in the offspring on PND 1, 7, 15, 23.

**Tissue collection**

Four sacrifice time-points were chosen throughout lactation in order to obtain a good coverage of the pup’s development. PND1 intended to examine the influence of an "overweight" or "lean" pregnancy on the pups' obesity levels at birth, but it was impossible to extract fat pads from such small and lean animals and only plasma and external parameters were collected. PND 6-7, 15-16 and 22-23 were chosen in order to characterize each week of the suckling period. On the experimental day, rats were weighed, measured and sacrificed between 11:00 AM and 2:00 PM. For tissue collection on PND1, all the litter was culled. For the rest of the ages, one male and one female were culled for tissue collection, always taking care that at least 7 pups remained in the litter. In order to be able to maintain large, similar-size litters after culling pups for tissue collection while reaching a reasonable number of subjects per measure,
we collected data from pups from a larger number of litters than the ones whose dams were studied during pregnancy and lactation. Thus, we also collected pups culled from additional litters (that were larger than 7 pups) in our breeding colony. Interscapular brown adipose tissue (BAT), retroperitoneal (Retro), inguinal (IAT) and epydidimal white fat (EPY) (only males) tissues were collected from decapitated animals, weighed and immediately frozen on dry ice. Measurable amounts of EPY tissue were only available at PND23. Inguinal white fat samples were preserved at -80 °C until analyzed. Trunk blood for leptin analysis was collected in chilled heparinized vacutainer tubes coated with EDTA. On PND1 plasma from 2-5 same-sex siblings was pooled in each tube. At all other ages N=1 per tube. Plasma was stored at -80°C until assayed.

**Leptin**

Plasma leptin levels were assessed using a commercial ELISA kit (R & D Systems, Minneapolis, USA) according to the manufacturers' instructions.

**Histology**

Samples of the inguinal white adipose tissue (I) were used for the characterization of the adipocyte cross sectional area. The parameters were measured by an observer blind to the experimental group. 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 X 200 magnification. For each inguinal fat pad examined, between 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 50 cells were analyzed. Representative cells chosen presented a smooth and clear membrane, with no granulation around. A similar methodological approach was described elsewhere (31, 49). The estimated number of cells per fat pad was calculated with the average cell diameter, a density conversion factor (0.915 g/cc), and the mass of the fat pads, as previously described (3, 32).

**Statistical analysis**

Maternal weight gain and intake data were analyzed by repeated measures analysis of variance (ANOVA) with strain (OLETF-LETO) as the independent variable and measurement day as the repeated measure. Group differences on each of the days of gestation and lactation were analyzed by one-way ANOVAs followed by post-hoc Scheffe tests. Offspring data were analyzed by univariate ANOVA with strain (OLETF-LETO), sex, and measurement day as the 3 independent variables. Significant interactions were followed up by post-hoc ANOVA within each group with Duncan's test for pairwise comparisons (P<0.05) or by comparisons of OLETF vs. LETO rats at particular ages.

**Results**

**Gestation**

As shown in Fig. 1, the patterns of BW change over gestation were similar, but significantly greater in OLETF compared to LETO dams (F(5,15)=12.58, p<0.001 - interaction effect). From GD10 and on, the percent of BW gain of the OLETF females was significantly greater than that of the LETO females (p<0.05). Furthermore, on postpartum day 1 OLETF dams still had BW levels
that were significantly higher than at gestation onset (p<0.001). In contrast, LETO dams returned to BW levels that were not significantly different from GD1 (Figure 1). Mean raw body weights are presented in Table 1. OLETF dams weighed significantly more than LETO controls over all points measured during gestation and on postpartum day 1 (F(1,19)=23.31, p<0.001 overall; p<0.05 at different days of comparison).

The patterns of caloric intake were significantly different among the 4 groups (F(44, 66)=8.14, p<0.001; Figure 2), with a significant main effect of strain (OLETF females ate significantly more than LETO females (F(3,37)=118.99, p<0.001)). On all 22 days of gestation, there were significant differences among the groups (p<0.01). Post-hoc Scheffe tests showed that control, non-pregnant OLETF rats consumed significantly more Kcal than control LETO rats. The OLETF pregnant dams ate consistently more than their within-strain non-pregnant controls starting from GD5, until GD20. Interestingly, pregnant LETO dams starting overeating later, from GD9 until GD20. The daily intake differences were significant on GD9, 10, 12, 16 & 17 (Scheffe tests). From GD20 until birth, pregnant females consumed similar amounts as controls. Pregnant OLETF dams ate significantly more than pregnant LETO controls on all gestation days (p<0.05) with two exceptions: GD20 & 22.

**Lactation**

The number of pups born to LETO (mean= 8, SEM= 0.73) and OLETF (mean= 10, SEM= 0.90) dams was not significantly different (t(19)=1.41, NS).

The patterns of BW change during the postpartum period were opposite and significantly different in the two strains (F(5,12)=8.30, p<0.001 - interaction effect), LETO dams significantly gained
weight (% of PND1 BW) from the earliest age measured, PND 5 and continued to do so (p<0.05). In contrast, the weight of OLETF dams significantly reduced decreased starting from PND 15 and on (p<0.05). Starting from PND10, strain differences in percent BW change were statistically significant (p<0.01) [Figure 3]. Mean raw body weights are presented in Table 1. OLETF dams weighed significantly more than LETO controls over all points measured during lactation (F(1,15)=26.07, p<0.001 overall; p<0.01 at different days of comparison, with one exception: p<0.05 on PND22, when strain differences were at minimum).

The pattern of food intake was significantly different among the 4 groups (F(66,40)=4.54, p<0.001 –interaction effect). A significant main effect of strain was found, with OLETF females consuming more Kcal than LETO females (F(3,34)=136.24, p<0.001). Control non-lactating groups showed stable levels of intake over the assessment period, while the lactating groups increased intake significantly over time (p<0.001). Post-hoc Scheffe tests showed that the two lactating groups ate significantly less than control non-lactating female rats on the day of birth (PND0). From PND1 and on, both lactating groups ate significantly more than LETO controls and from PND4 and on, significantly more also than non-lactating OLETF controls (Fig. 4). Intake of lactating rats from both strains did not differ significantly over the 23 days of assessment. We would like to point out that the intake levels presented here in the third postnatal week include both the dams' and the pups', because they shared the source of food from the emergence of independent ingestion, and over the third week of lactation the pups increasingly feed independently.

**Pups**
OLETF and LETO pups’ weight differed significantly across time points (F(3,233)=15.06, p<0.001, for strain X age interaction effect), irrespective of the sex of the pups (sex main effect and interactions with sex, NS). At all ages and within each sex, OLETF pups weighed significantly more than LETO pups (p<0.01) [Fig. 5].

The weight of the excised white fat pads (expressed as percent of the rat's BW) was significantly greater overall in OLETF vs. LETO rats (F(1,91)=121.38, p<0.001), in females vs. males (F(1,91)=5.12, p<0.05), and in 7 day-old vs. 15- and 22-day old rats (F(2,91)=17.96, p<0.001; post-hoc Duncan's test (p<0.05). Furthermore, LETO pups had significantly less white fat on PND 15, compared to older and younger ages; and 7-day-old OLETF pups had significantly higher levels of white fat compared to older OLETFs (PND 15 and 22; F(2,91)=13.88, p<0.001, for the strain X age interaction). Other interactions were not statistically significant (see Fig. 6).

Group differences in different fat pads (expressed as percent of BW) are presented in Table 2. **Inguinal adipose tissues**: Inguinal fat as a percentage of BW was significantly greater, in OLETF compared to LETO pups at all ages, without sex differences. The only exception was at PND23; at this age, female OLETF rats had more IAT than male OLETFs (F(3,24)=15.57, p<0.001 on PND7; F(3,38)=24.50, p<0.001 on PND15; F(3,33)=9.20, p<0.001 on PND23).

**Retroperitoneal fat pads**: On PND 7 and 15, Retroperitoneal fat pads were heavier in OLETF than in LETO pups, with no sex differences (F(3,24)=12.44, p<0.001 on PND7; F(3,35)=14.41, p<0.001 on PND15). On PND23, there were no group differences (NS).

**Brown adipose tissue**: On PND7, brown adipose fat pad was significantly larger in OLETF than in LETO pups, with no sex differences (F(3,24)=4.97, p<0.01); on PND15 there were no significant
group differences; while on PND23, LETO males had significantly more BAT than OLETF males (F(3,33)=4.09; p<0.05; the difference in the females was not significant).

**Adipocyte size and number**

Mean inguinal adipocyte size was greater in OLETF than in LETO pups (F(1,41)=54.97, p<0.001), males had larger adipocytes than females on PND22 but not earlier (F(1,41)=7.27, p<0.001 for sex main effect; F(2,41)=3.97, p<0.05 for the sex X age interaction) and this was true in both LETO and OLETF strains (P<0.05, see Fig. 7). The estimated adipocyte number was larger in OLETF compared to LETO pups (F(1,41)=9.0, p<0.01) and in females compared to males (F(1,41)=24.81, p<0.001)(sex main effect), with a significant sex X age interaction (F(2,41)=3.86, p<0.05). Estimated adipocyte number increased across development in females of both strains, while males' adipocyte number remained almost constant. Post doc Duncan's tests revealed that females had a greater estimated adipocyte number on PND7 and 22 in the LETO strain and at all ages in the OLETF strain (p<0.05), compared to males. Significant strain effects were only observed between males on PND7 (Fig. 8).

**Leptin**

OLETF pups had significantly higher leptin levels on PND7, compared to LETO pups, within each sex; on the other days the differences were not significant (F(3,52)=12.38, p<0.001, for the strain X age interaction). However, the strain difference on PND1 is based on samples pooled from several newborn pups, thus the total N is greater than the number of samples analyzed. T-tests within each sex showed that male and female OLETF newborns had higher levels of leptin at this age, compared to same-sex LETO newborns (p<0.05), see Fig. 9). Overall, leptin levels were higher on PND7 and 22, compared to 1 and 15 (F(3,52)=11.14, p<0.001).


**BMI and Waist circumference**

Group differences in BMI were as follows: on PND1, LETO females presented lower BMI than OLETF females (in the males the strain difference was not significant) and on PND 15 LETO males presented lower BMI than the OLETF males (in the females the strain difference was not significant). On PND7 no significant differences were observed; while at weaning age, a significant strain effect (OLETF>LETO) was found in both sexes (F(3,109)=3.68, p<0.05, Fig. 10).

Regarding waist circumference, LETO females presented lower WC than OLETF females on PND1 (in the males the strain difference was not significant); from PND7 on a significant strain effect was found in both sexes with OLETF rats presenting consistently greater WC than LETO controls (F(3,109)=22.86, p<0.001, for the strain X age interaction; Fig. 11).

**Discussion**

In the present study, we explored the short-term consequences of maternal obesity during pregnancy and lactation on several measures of adiposity and growth in infant OLETF and LETO males and females. In the last few years, researchers have focused their attention on the consequences of maternal obesity on food preferences and long-term obesity levels of the offspring, mostly using rodent animal models. In these studies, females are usually given access to high fat or highly palatable foods in order to induce obesity and/or hyperphagia. The OLETF rats' development of obesity is secondary to their hyperphagia (34), which is a direct consequence of their genetic mutation. Therefore, OLETF females (and males) are obese and hyperphagic even when consuming regular chow. Some studies have shown that giving the OLETF rats access to...
high fat or palatable food leads to an even more morbid obesity (9, 26) but this is not necessary to induce moderate obesity in this strain. Therefore, the results presented here are related to the hyperphagia and obesity levels of the dam during pregnancy and lactation (and to the pups' genetic tendency) and not to any specific kind of "junk food".

OLETF dams were obese at the time they became pregnant, and exhibited hyperphagia resulting in significantly higher rates of weight gain than controls during gestation. It should be mentioned, that OLETF females weighing between 23-30% more than controls were primarily used for these studies. At this level of obesity they became pregnant, while OLETF females weighing more than that usually did not. Moreover OLETF females in this study were on average younger (8-12 weeks old) than LETO females (8-16 weeks of age), because only the youngest became pregnant and gave birth to the expected number of pups.

OLETF females were hyperphagic compared to LETO females during pregnancy, but this difference was not maintained after delivery. Lactating OLETF dams consumed the same amounts of food as lactating LETO dams [note though that a portion of the intake recorded in the third postnatal week in both strains was pup-consumption, increasing as the offspring matured towards weaning age]. Interestingly, while OLETF dams gained significantly more weight during pregnancy, the lactating period represented a time point where OLETF females gradually lost weight, almost reaching weight levels of LETO dams at the time of weaning. We have recently reported that this weight loss throughout lactation is accompanied by a decrease in fat stores, adipocyte cell size and leptin levels (49). We can only speculate about why OLETF females do not maintain a relative hyperphagia beyond that shown by lactating controls during such an energy-
demanding period. It appears that overall; the OLETF strain has difficulties in compensating to energy challenges. OLETF rats do not compensate by reducing their intake of standard chow after intake of a palatable food (43). OLETF pre-weanling pups do not reduce their milk intake in independent ingestion tests after a fat preload (13). There is the same lack of compensation to increased energy demands: Male and female OLETF do not increase their intake to compensate for increased energy expenditure when they exercise while LETO females do (11, 42). While the OLETF females in the current study were hyperphagic compared to non-lactating OLETF females, the relative (normalized to body weight) degree of increase was not as great as that found in lactating LETO females. This difference, together with the demands of rearing hyperphagic pups, may account for their weight loss. A higher energy investment in milk production may also contribute to the weight loss of OLETF females during lactation. This is an issue still to be elucidated.

As we have already reported, OLETF pups are born significantly heavier than controls (39). This might be a consequence of the exposure to an obese dam during pregnancy, the genetic mutation itself, or both. In the present study, we characterized their obesity development and identified pre-obese features in this strain that suggest that this disorder develops early in life in the OLETF strain, making them a valid model of childhood-onset obesity.

During lactation, the increased amount of nursing and consequently of milk that the OLETF pups receive from their obese mother, may be at least partially responsible for the high body fat percentages observed during suckling. As early as PND7, OLETF pups had increased WC, fat levels and larger adipocytes. These differences between the strains remained throughout lactation
in both sexes. However, at the end of the 2nd PN week (PND15) there was an obvious decrease in body fat and leptin levels in the OLETF strain. At this point, OLETF pups also show a temporary lack of increase in adipocyte hypertrophy. We have previously studied each PN week separately and the 2nd PN week frequently failed to present differences between the strains in maternal and nursing behavior (39) and sometimes also in pup suckling characteristics (42). It seems that this PN week represents a "transition time", where the very significant differences observed in the first and 3rd PN weeks become moderate. This 2nd PN week appears to be a period of time where differences between the strains diminish, and could therefore be an appropriate target for future interventions.

The profile of ontogenetic changes in BAT (levels decreasing) vs. WAT (increasing) found in this study, is consistent with previous descriptions in other strains (17). It is interesting that the BAT was significantly larger in OLETF pups compared to controls only on PND7. BAT has been reported to become hypertrophic under several conditions, with overfeeding being one of them (4, 25, 37). At this age, OLETF pups also presented greater white fat percentages and adipocytes and higher leptin levels compared to controls, a profile resembling adult-like differences. In previous studies, hyperphagic characteristics were systematically observed in the pre-obese strain around this age, both in independent ingestion tests and nursing (with increased suckling rate) (12, 41). Nevertheless, changes in BAT weight do not necessarily reflect changes in its activity or in mitochondrial thermogenic capacity (16, 46). By PND15, BAT in the OLETF pups was normalized to LETO levels. Thus it would appear that this transitory neonatal increase in BAT may be in response to the high amounts of food consumed in the first postnatal week.
The BMI and waist circumference measurements were added as non-invasive parameters for identifying further obesity features, as these are similar to measures regularly used in humans. In the case of BMI, it seems that even when body weight and fat are relatively high in the pups, this is not always reflected by this parameter. The classic BMI index also fails to identify obesity in children and teenagers in a reliable manner because of their constant physiological changes; and in order to correctly diagnose overweight in that population (under 20 years of age), the original BMI index was modified by including sex and age parameters together with BW and height (18). While the original BMI appears to be an effective tool for identifying obesity levels in adult rats (2), an improved index has not yet been created for young animals, making it a less accurate method for diagnosing obesity in rat pups. In contrast, measuring waist circumference appears to be a more effective way of detecting the different pattern of adiposity development taking place in the OLETF pups as early as PND7 in both sexes.

Females of both strains, although weighing less, exhibited greater percentages of body fat than the males, and this is particularly true for the OLETF females. Interestingly, this finding is not accompanied by adipocyte hypertrophy, with OLETF females presenting smaller adipocytes than OLETF males on PND15 and 22. The analysis of adipocyte number estimated the presence of more cells at these ages in both strains, suggesting that adipocyte hyperplasia is more pronounced in the females than in the males in the pre-weaning period. In rats, the phase of highest hyperplasia occurs between birth and PND40 and from then and on cell number remains more or less constant. From PND40 until PND80, adipocyte hypertrophy is the main process responsible for the growth of the fat pads (24). Unlike many tissues, adipose tissue has the potential for almost unlimited growth, but importantly, diet-induced increases in fat cell number are apparently irreversible (19,
22). The comparison between OLETF and LETO rats shows a marked hypertrophy of the fat cells of the inguinal fat pad at all these early ages in the pre obese OLETF in both sexes. Adipocyte hypertrophy has also been reported in weanling rats from dams fed a highly palatable diet in pregnancy and lactation (8) and in pups that underwent postnatal overfeeding due to a reduction in litter size (21). One of the few studies performed on adipose tissues of developing rats further reported a general pattern of increase in fat cell size in males and increased fat cell number in females as a consequence of postnatal overfeeding (20). A high amount of fat cells may be an important determinant responsible for the differences in fat depot sizes observed in the (obese) females compared to the males. Overall, it seems that obesity development in the OLETF strain undergoes similar paths in both sexes regarding adipocyte hypertrophy, but sharply different paths regarding adipocyte hyperplasia, at least in the pre-weaning period. It appears that, while OLETF males' increase in fat levels relies mainly on exaggerated fat cell enlargement, OLETF females present both high increase in cell number and size, predisposing them to a potentially worse profile of long-term obesity.

The profile of decreases in leptin levels, cell size and fat percentages observed from PND7 to 15 in the OLETF strain in both sexes, is similar to what has been previously described in another model of early-onset obesity; where litter size was reduced to 4 in order to induce early obesity appearance in the offspring (21). Here, early obesity was characterized by adipocyte hypertrophy during the first 10 days of life and by a combination of adipocyte hypertrophy and hyperplasia from then on. Even though that study did not differentiate between males and females during the suckling period, their results appear to be in accordance with ours, with a sharp transitory increase in cell size (and in leptin and % fats) during the first phase of the lactating period as a consequence
of early overeating, which was followed by an hyperplastic and hypertrophic period until adolescence. The significant surge in leptin levels observed around PND 7 both in male and female OLETF pups may also be relevant to long term obesity risk. In a study performed on mice, neonatally leptin-treated offspring exhibited an impaired response to acute peripheral leptin administration on a regular chow diet at around 8 weeks of age, with impaired leptin transport to the brain as well as an increased density of hypothalamic nerve terminals (48). While OLETF females’ sensitivity to leptin has never been examined, adult OLETF males have been reported to be resistant to peripheral (but not i.c.v.) administration of leptin already at 8 weeks of age. (35). Whether this is a consequence of this early leptin surge or of their overall high leptin levels remains to be elucidated.

In sum, OLETF dams are hyperphagic and obese during pregnancy, leading to overweight pups at the time of birth. According to an investigation recently published, this alone might be enough to ensure an overweight adulthood (44). During lactation, OLETF dams eat like normal LETO lactating females, but in contrast to the weight gain observed in the LETO strain; normal intake leads OLETF females to gradually lose weight. The gradual increase in the nutritional demands of the OLETF pups (reflected by increased nursing time and initiative, especially in the 3rd PN week (40, 41), is accompanied by a sharper body weight loss in the dams. It appears that the pups consume their dams' fat stores beyond the dam's normal levels, because the same energy intake in the LETO dams leads to transitory body weight gain during lactation and normal weighing pups. A cross-fostering study currently being performed will shed light on whether raising pups of the opposite strain will have different consequences on the obesity levels of the dams or the pups
during lactation and will help with the understanding of critical periods of obesity development in this strain.

*Perspectives and Significance:* OLETF rats present pre-obese characteristics during the suckling period beyond abnormal eating behavior, such as increased body fat, larger adipocyte size and number (in the females) and waist circumference and develop obesity as a consequence of chronic hyperphagia. On the basis of these new physiological and the previous behavioral findings, we propose the OLETF strain as a valid genetic model of childhood-onset obesity.
Acknowledgements

We thank Dr Kawano of the Tokushima Research Institute (Otsuka Pharmaceutical, Japan) for the generous gift of the OLETF and LETO rats.

This work was supported by the US-Israel Binational Research Foundation (AW and THM).

A portion of this research was presented at the 13th Annual Meeting of the Society for the Study of Ingestive Behavior, Pittsburgh, PA, USA.
References


Figure legends

Fig. 1:
Percentage body weight change in OLETF and LETO pregnant females from the day of conception. Data are presented in means and SEM. *p<0.05, **p<0.01, ***p<0.001 (for differences between the strains at each GD) and ### p<0.001 (significant difference in % BW retention compared to GD0 within the OLETF strain). N= 9 LETO, 12 OLETF.

Fig. 2:
Daily intake of OLETF and LETO pregnant and control females from the day of conception until parturition. Intake is presented in Kilo-calories. Data are presented in means and SEM. * p<0.05, for OLETF pregnant vs. LETO pregnant; # p<0.05, ## p<0.01 and ### p<0.001, for LETO pregnant vs. LETO control and ++ p<0.01 for OLETF pregnant vs. OLETF control. There was also a significant effect between OLETF and LETO controls (p<0.05) not noted in the figure due to lack of space. N= 9 LETO, 12 OLETF.

Fig. 3:
Percentage body weight change of lactating OLETF and LETO females from birth until weaning. Data are presented in means and SEM. **p<0.01, ***p<0.001, for differences between the strains at each PND; #p<0.05, ##p<0.01, ###p<0.001 for the differences in body weight change compared to PND1 within the LETO strain; and +p<0.05, ++p<0.01 for the differences in body weight change compared to PND1 within the OLETF strain. N= 9 LETO, 8 OLETF.
**Fig. 4:**

Daily intake of lactating OLETF and LETO females from birth until weaning and same aged OLETF and LETO controls. Intake is presented in Kcal. Data are presented in means and SEM. * significantly different from both non-lactating groups. # significantly different from the LETO non-lactating group. N= 9 LETO, 8 OLETF.

**Fig. 5:**

OLETF and LETO pups’ body weight in grams on PND1, 7, 15 and 23. Data are presented in means and SEM. **p<0.01, ***p<0.001 for the females, ## p<0.01, ### p<0.001 for the males (for strain differences at this age). N= 10-22 per group.

**Fig. 6:**

Total white fat (expressed as percent of BW) of OLETF and LETO males and females on PND7, 15 and 23. Data are presented in means and SEM. ***p<0.001 for the females, ## p<0.01, ### p<0.001 for the males (for strain differences at each age). N= 6-14 per group.

**Fig. 7:**

Adipocyte size of OLETF and LETO males and females on PND7, 15 and 23. Data are presented in means and SEM. *p<0.05, ***p<0.001 for the females, # p<0.05 for the males (for strain differences at each age). N= 4-6 per group.

**Fig. 8:**

Estimation of the inguinal adipocyte number of OLETF and LETO males and females on PND7, 15 and 23. Data are presented in means and SEM. + p<0.05, +++ p<0.001 for LETO females vs. LETO males, # p<0.05 for OLETF females vs. OLETF males, and *p<0.05, for strain differences between the males. N= 4-6 per group.
**Fig. 9:**

Plasma Leptin levels of OLETF and LETO males and females on PND1, 7, 15 and 23. Data are presented in means and SEM. *p<0.05, ***p<0.001 for the females, # p<0.05 for the males (for strain differences at each age). N= 4-7 per group.

**Fig. 10:**

Body mass index (BMI) of OLETF and LETO males and females on PND1, 7, 15 and 23. Data are presented in means and SEM. *p<0.05, ***p<0.001 for the females, # p<0.05, ### p<0.001 for the males (for strain differences at each age). N= 6-10 per group.

**Fig. 11:**

Waist circumference of OLETF and LETO males and females on PND1, 7, 15 and 23. Data are presented in means and SEM. **p<0.01, ***p<0.001 for the females, ### p<0.001 for the males (for strain differences at this age). N= 6-10 per group.
Table 1.

Raw body weight (grams) of OLETF and LETO females throughout pregnancy and lactation.

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<tr>
<th></th>
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<tr>
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Notes: Data are presented in means and SEM. N= 9 LETO, 8(lactation)-12(pregnancy) OLETF.

*p<0.05, **p<0.01 and ***p<0.001 for differences between the strains.
Table 2.

Weight of different fat pads collected from the pups (expressed as percent of BW).

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Notes: IAT: Inguinal adipose tissue, Retro: Retroperitoneal adipose tissue, BAT: Brown adipose tissue. M: males, F: females. Data are presented in means and SEM. * Significantly different from same-sex and same-age LETO controls. # Significantly different from the same-strain opposite sex. All significant differences derived from post-hoc Duncan's tests, p<0.05. N=6-14 per group.