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Responses of the Adult Rat Glucose Metabolism to Early Life Feeding, Caloric Restriction and Refeeding

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Abstract: Early life overfeeding in the rat can be experimentally induced by reducing litter size. This investigation assessed the consequences of this manipulation on glucose metabolism *in vivo* and in isolated hepatocytes in 150-day old rats. Additionally, after body growth, the effects of caloric restriction and refeeding were tested. Adult rats from control (G9) and reduced litters (G3L) did not differ in body and fat weights, glucose tolerance or insulin resistance (insulin-induced hypoglycemia), or hepatocyte glucose release under basal or gluconeogenic conditions. Caloric restriction (G3R) reduced body and fat weights, decreased glucose decay after insulin injection and decreased hepatocyte gluconeogenic glucose release. Refeeding after caloric restriction reversed these parameters to those of the freely-fed groups (G9 and G3L). Taken together, these results suggest that the liver glucose metabolism is not programmed by lactational overfeeding, but rather is responsive to the current nutritional condition of the animal.

Key words: Glucose homeostasis, metabolic programming, reduced litter, caloric restriction, refeeding.

1. Introduction

In the rat, reducing litter size during lactation is a classical model leading to adult obesity [1-4]. It is reported that overfeeding in early life programs later life obesity by modulating the hypothalamic circuits that control food ingestion, energy expenditure and metabolism, which in the rat are susceptible to environmental conditions during late gestation and lactation [1, 2]. As a central organ of energy metabolism, the liver is a potential target of these early life events. Glucose homeostasis is reported to be compromised in adult life after perinatal overfeeding [3, 4] and a role for the liver should be expected.

Moderate caloric restriction may overcome the negative effects of obesity and its comorbidities [5-7]. In previous investigations, reduced-litter rats were subject to caloric restriction soon after weaning, and it had the expected result on body growth and composition. However, in those young adult rats (up to 90 days old), litter size did not overtly influence liver metabolism or whole-body glucose homeostasis, while caloric restriction had much more pronounced effects.

As in these previous studies caloric restriction was imposed during the period of body development, the alterations could have been the consequence of blunted growth rather than from decreased food intake *per se*. Therefore, this investigation was designed to complement those other investigations and answer the following questions: Is liver glucose metabolism in the grown adult rat programmed by early life nutritional conditions? To what point systemic glucose changes, if they appear, can be ascribed to the liver? After body growth is complete, can caloric restriction reverse any changes caused by early life overfeeding? If so, is this

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reversal resistant to refeeding?

2. Method and Materials

2.1 Ethical Approval

The international ethical guidelines on animal care and experimentation were followed. All the experimental procedures were approved by the Ethics Commission of the Institution (certificate 1720290116).

2.2 Experimental Groups

Wistar *Rattus norvegicus* albino rats were used. Pregnant females were placed in individual cages in an animal house of controlled temperature (22 ± 2 ^{o}C) and light/dark cycles (12 h light/12 h dark). Water and rodent chow were supplied *ad libitum*.

One day after birth, the litters were organized so that each dam had either nine or three male pups, thus establishing the initial experimental groups, control-litter (G9) and reduced-litter (G3) groups, respectively. Control litter size was based on the average number of pups born per litter of the original rat colony.

At weaning (21 days old), the pups were placed in plastic boxes (three rats/box). Group G9 (n = 20) was given water and chow *ad libitum* until the age of 150 days. Group G3 was fed at will until 60 days of age, when it was subdivided into the following groups:

G3L (n = 20): animals that were fed at will until the age of 150 days;

G3R (n = 25): animals that had their supply of chow reduced in 30% relative to the intake of the G9L, corrected for body weight, from 60 to 150 days of age;

G3RL (n = 24): animals that had their supply of chow reduced in 30% relative to the intake of the G9L, corrected for body weight, until the age of 90 days; refeeding at will was then allowed until these rats were 150 days old;

Body weight and naso-anal length were measured at the ages of 60, 90 and 150 days. These were used to calculate the body mass index for rats (BMI, g/cm^2) [8, 9].

At the age of 150 days, some of the rats of each group were used for the *in vivo* procedures described below. Immediately after each test, the animals were returned to their boxes and given water and chow. Euthanasia was carried out a few days later. Other rats of each group were used for *in vitro* protocols (hepatocyte isolation and incubation). All the procedures were carried out after overnight fasting (approx. 14 h).

Euthanasia was performed by intraperitoneal injection of excess anesthetic (thionembutal 120 mg/kg body weight plus lidocaine 5 mg/kg). Blood was rapidly collected, centrifuged and stored at -80 ºC for further analytical determinations. Fats and liver were removed and weighed.

2.3 Intravenous Glucose Tolerance Test (ivGTT)

Under anesthesia (thionembutal 40 mg/kg body weight plus lidocaine 5 mg/kg, ip), a heparinized cannula (50 IU heparin; 1 mL NaCl 0.9%) was implanted into the right jugular vein and attached to the dorsal cervical region. After overnight fasting (about 12 hours) a bolus of glucose (1 g/kg body weight, dissolved in saline) was infused through the cannula. Samples of blood were collected immediately before glucose infusion (0 min) and 5, 15, 30, 45 and 60 min after glucose infusion. Blood glucose was determined with test strips and glucometer (Optium Exceed®; Abbott, São Paulo-SP, Brazil). The AUC (area under curve) of blood glucose variation was calculated using blood glucose at 0 min as baseline [10].

2.4 IIH (Insulin-Induced Hypoglycemia)

The rats were ip injected with regular insulin (1 U/kg body weight; Novolin®; Novo Nordisk, Montes Claros-MG, Brazil). Blood samples were collected from the tail just before insulin injection (0 min) and at 5, 10, 15, 20, 25, 30, 60, 120, 180, 240 and 300 min. Blood glucose was determined with test strips and glucometer (Optium Exceed®). The index of glucose decay (kITT, %/min) was calculated for the first 30 min

of the IIH [11].

2.5 Determination of Plasma Insulin

Plasma insulin was measured by radioimmunoassay in gamma counter (Wizard2 Automatic Gamma Counter®, TM-2470, PerkinElmer, Shelton-CT, USA). Human insulin was used as standard along with an anti-rat insulin antibody (Sigma-Aldrich, St. Louis-MO, USA) and ^{125}I-labeled recombinant human insulin (PerkinElmer). The intra-assay coefficients of variation were in the range of 8-10%.

2.6 Incubation of Hepatocytes

After overnight fasting, the rats were anesthetized with thionembutal (40 mg/kg body weight plus 5 mg/kg lidocaine, ip). The liver was perfused through the portal vein for 15 min with non-recirculating aerated calcium-free KH (Krebs-Henseleit) buffer (pH 7.4, 37 ºC). Next, KH containing calcium and collagenase (700 U/dL, pH 7.4) was perfused in a recirculating system for 5-7 min. The liver was removed, manually fractioned, filtered and centrifuged three times (4 ºC, 530 rpm) with albumin-containing KH (0.2 g/dL) [9, 11-13]. Samples of 10^6 cells/mL were incubated with a gluconeogenic precursor (glycerol or lactate or alanine or glutamine) at the concentration of 5 mM each [9, 11] in a water bath for 60 min under constant agitation and aeration. Glucose and urea release under these conditions were termed gluconeogenic release. Additional flasks containing only the KH buffer were used to determine the control (basal) release of the products analyzed. After incubation, the samples were centrifuged for 10 min at 3,000 rpm (room temperature) and the supernatant stored for biochemical assays.

2.7 Biochemical Assays

Commercial kits (Gold Analisa®; Belo Horizonte-MG, Brazil) were used for the biochemical determinations from the plasma samples (total and HDL cholesterol, triglycerides and glucose) and from the supernatants of the hepatocyte incubation (glucose and urea).

2.8 Statistical Analyses

For the sake of consistency, all data sets in tables and figures are shown as mean ± SD. The number of replicates for each data set is indicated.

Shapiro-Wilk and Kolgomorov-Smirnoff normality tests were applied. Student's test t was used for comparisons of data sets from two groups, or two time-points within the same group. One-way analysis of variance (ANOVA) followed by Tukey's *post-hoc* test was employed to compare three or more data sets. The significance level adopted was 5%. The statistical analyses and the construction of the figures were made on Prism® 5.0 (GraphPad, San Diego-CA, EUA).

3. Results and Analysis

Table 1 brings the body weight, BMI and blood glucose of the groups at the ages of 60, 90 and 150 days. Tissue weights at 150 days are also shown. Group G3L had higher body weight, BMI and blood glucose than the G9 at 60 days of age, but not at later ages. Food restriction from 60 to 150 days of age (G3R) decreased body weight, BMI and blood glucose, while refeeding from 90 to 150 days of age (G3RL) restored these values to the G9 and G3L levels.

Food restriction (G3R) markedly decreased the relative weight of all body fats and increased the relative liver weight. Abdominal fats and liver of the refed group (G3RL) were statistically similar to those of groups G9 and G3L, but mean values were higher (from 6% for epidydimal fat to 24% for mesenteric fat; 15% for liver). Subcutaneous fat weight of group G3RL, on the other hand, was four times higher than in group G3R, but 50% lower than in groups G9 and G3L.

The plasmatic profile of the groups at the age of 150 days is given in Table 2. Group G3L had higher TGL and VLDL than group G9. In the food-restricted rats (G3R), the lipid values were similar to those of the control, except for VLDL, which was even lower. On

Table 1 Biometric parameters, fasting blood glucose and tissue weights of rats from control (G9) or reduced litters (G3) fed at will (G3L), food-restricted in 30% (G3R) or refed after food restriction (G3RL), at the ages of 60, 90 and 150 days.

60 days	G9 (n = 11)		G3L (n = 13)	
Body weight (g)	230.3 ± 11.17		284.6 ± 17.62[a]	
BMI (g/cm^2)	0.52 ± 0.02		0.62 ± 0.04[a]	
Glucose (mg/dL)	76.55 ± 4.63		83.92 ± 9.82[a]	
90 days	G9 (n = 6)	G3L (n = 8)	G3R (n = 9)	
Body weight (g)	349.2 ± 13.00	345.9 ± 15.47	272.4 ± 23.37[a,b]	
BMI (g/cm^2)	0.61 ± 0.02	0.63 ± 0.03	0.59 ± 0.04[b]	
Glucose (mg/dL)	77.17 ± 5.57	71.38 ± 6.30	69.22 ± 4.92[a]	
150 days	G9 (n = 12)	G3L (n = 14)	G3R (n = 17)	G3RL (n = 16)
Body weight (g)	440.2 ± 34.16	444.2 ± 34.89	215.9 ± 31.66[a,b]	432.6 ± 33.45[c]
BMI (g/cm^2)	0.69 ± 0.03	0.70 ± 0.02	0.45 ± 0.07[a,b]	0.71 ± 0.06[c]
Glucose (mg/dL)	91.83 ± 8.44	96.35 ± 11.48	85.71 ± 8.84[b]	90.38 ± 6.04
Retroperitoneal fat (g%)	1.46 ± 0.19	1.56 ± 0.35	0.04 ± 0.03[a,b]	1.73 ± 0.46[c]
Epidydimal fat (g%)	1.41 ± 0.24	1.49 ± 0.14	0.14 ± 0.09[a,b]	1.56 ± 0.28[c]
Mesenteric fat (g%)	0.77 ± 0.19	0.86 ± 0.22	0.12 ± 0.06[a,b]	1.07 ± 0.40[c]
Visceral fat (g%)	3.73 ± 0.63	3.97 ± 0.60	0.37 ± 0.10[a,b]	4.04 ± 0.67[c]
Subcutaneous fat (g%)	1.50 ± 0.20	1.39 ± 0.20	0.20 ± 0.06[a,b]	0.80 ± 0.12[a,b,c]
Liver (g%)	3.36 ± 0.28	3.22 ± 0.24	4.00 ± 0.55[a,b]	3.72 ± 0.16[c]

Data shown as mean ± SD; [a] $p < 0.05$ *vs.* G9; [b] $p < 0.05$ *vs.* G3L; [c] $p < 0.05$ *vs*. G3R; Student's t-test for groups at 60 days of age; ANOVA-Tukey for groups at 90 and 150 days of age.

Table 2 Plasmatic parameters of 150-day old rats from control (G9) or reduced litters (G3) fed at will (G3L), food-restricted in 30% (G3R) or refed after food restriction (G3RL).

	G9 (n = 5)	G3L (n = 5-8)	G3R (n = 5-7)	G3RL (n = 5-8)
Total Chol (mg/dL)	78.38 ± 8.28	70.63 ± 18.98	67.36 ± 7.39	99.56 ± 9.15[b,c]
HDL-Chol (mg/dL)	39.50 ± 5.12	25.94 ± 10.54[a]	29.71 ± 8.30	36.00 ± 4.33
VLDL (mg/dL)	4.85 ± 0.93	22.75 ± 13.66[a]	4.41 ± 0.89[b]	28.55 ± 8.72[a,c]
LDL (mg/dL)	28.78 ± 7.86	21.94 ± 12.17	33.23 ± 5.65	35.01 ± 7.86[b]
TGL (mg/dL)	24.25 ± 4.63	113.8 ± 68.32[a]	22.07 ± 4.47[b]	142.8 ± 43.60[a,c]
Total proteins (g/dL)	6.42 ± 0.60	6.28 ± 0.05	5.03 ± 0.31[a,b]	5.53 ± 0.66[a]
Insulin (pg/mL)	17.00 ± 1.41	16.5 ± 1.92	28.25 ± 5.06[a,b]	35.00 ± 4.97[a,b]
Atherogenic index	1.85 ± 0.13	3.20 ± 0.97[a]	2.39 ± 0.59	2.79 ± 0.28[a]

Atherogenic index = Total Chol/HDL-Chol. Data shown as mean ± SD; [a] $p < 0.05$ *vs.* G9; [b] $p < 0.05$ *vs.* G3L; [c] $p < 0.05$ *vs.* G3R; ANOVA-Tukey.

the other hand, after refeeding (G3RL), total cholesterol, TGL, VLDL and LDL had values even higher than those of group G3L. Plasma proteins (Table 2) were decreased by food restriction (G3R) and did not increase significantly after refeeding ($p > 0.05$, G3RL *vs.* G3R). Insulin was significantly higher in reduced-litter rats under caloric restriction (G3R) or refed (G3RL). The atherogenic index was higher in groups G3L and G3RL compared to group G9.

The profile and AUC of the ivGTT are in Fig. 1. Five min after glucose infusion, blood glucose reached values of 300 mg/dL or higher in all the groups. At this time, group G3L had the highest blood glucose, while group G3R had the lowest. By the end of the test (60 min), blood glucose returned to basal (0 min) values in all the groups. Although blood glucose at 0 min was lower in groups G3R and G3RL than in group G3L, it was not different from the 60 min value of the group. The AUC did not differ between the groups.

Fig. 2 is the profile and kITT of the insulin-induced hypoglycemia (IIH). At 10 min, blood glucose was significantly lower than at 0 min in every group. The

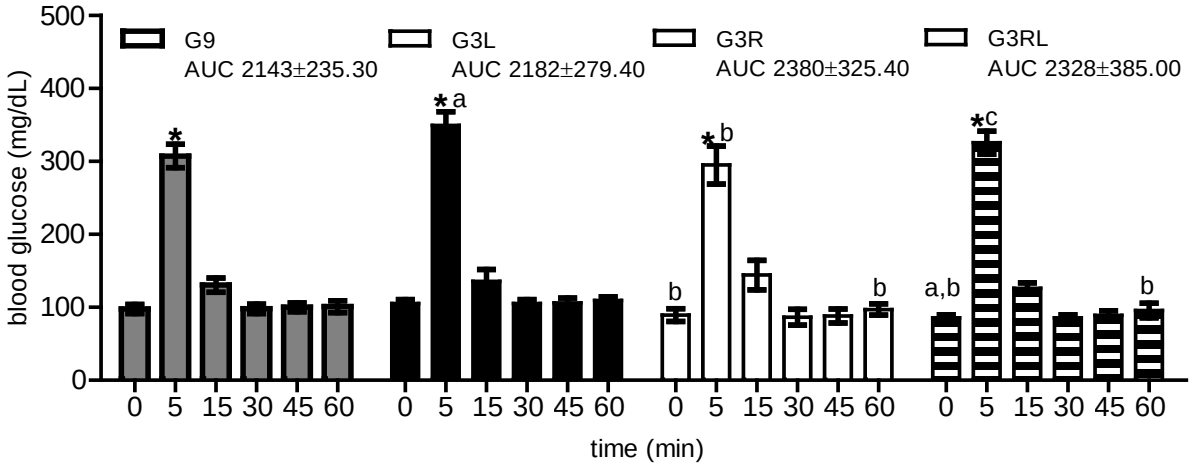

Fig. 1 Profile and AUC of intravenous glucose tolerance test (ivGTT) of 150-day old rats from control (G9) or reduced litters (G3) fed at will (G3L), food-restricted in 30% (G3R) or refed after food restriction (G3RL).
Data shown as mean ± SD; n = 5-7 per group. [a] $p < 0.05$ *vs.* G9; [b] $p < 0.05$ *vs.* G3L; [c] $p < 0.05$ *vs.* G3R; ANOVA-Tukey.* $p < 0.05$ *vs.* time 0 min of the group; Student's t-test.

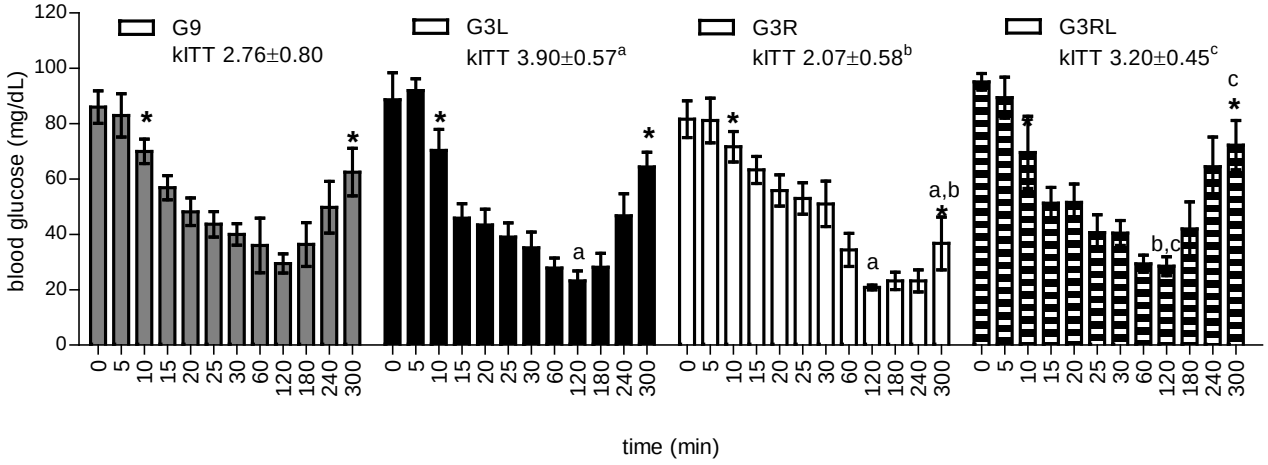

Fig. 2 Profile of insulin-induced hypoglycemia (IIH) and kITT of 150-day old rats from control (G9) or reduced litters (G3) fed at will (G3L), food-restricted in 30% (G3R) or refed after food restriction (G3RL).
Data shown as mean ± SD; n = 6-9 per group. [a] $p < 0.05$ *vs.* G9; [b] $p < 0.05$ *vs.* G3L; [c] $p < 0.05$ *vs.* G3R; ANOVA-Tukey.* $p < 0.05$ *vs.* time 0 min of the group; student's t-test.

lowest blood glucose was recorded 120 min after insulin injection. At this time, blood glucose was lower in groups G3L and G3R than in the control (G9) and refed (G3RL) groups.

None of the groups recovered completely from the hypoglycemic episode at 300 min. Recovery was significantly lower in group G3R, where blood glucose at 300 min was less than 40 mg/dL. At this moment, blood glucose of the other groups was at least 60 mg/dL.

Blood glucose decay was faster in group G3L (as indicated by the higher kITT), but it was markedly slower in group G3R.

Fig. 3 is the glucose release by isolated hepatocytes incubated under basal condition or with the gluconeogenic precursors glycerol (gly), lactate (lac), alanine (ala) or glutamine (gln). Basal glucose release was significantly higher in the G3R and lower in the G3RL when compared with groups G9 and G3L. Glucose release under gluconeogenic conditions, on

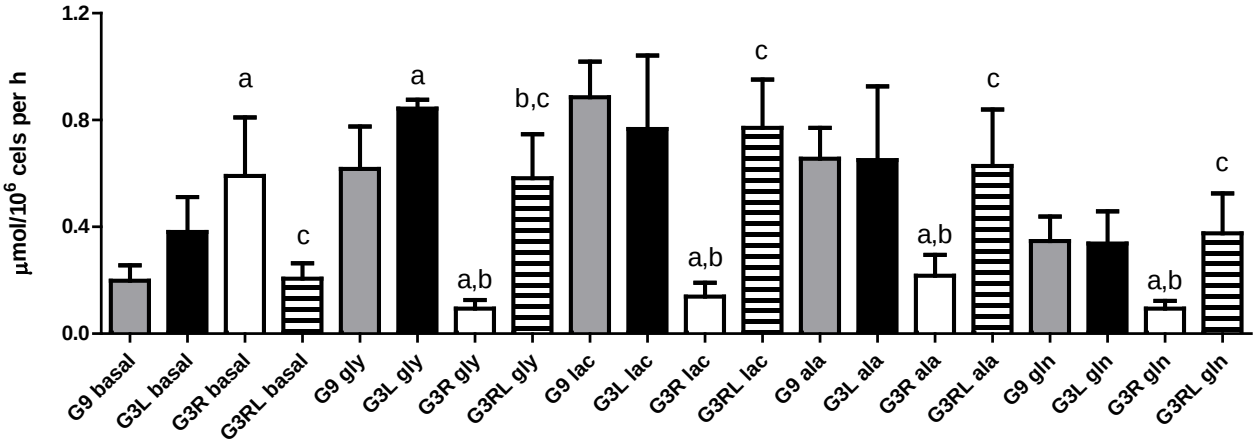

Fig. 3 Glucose release by incubated hepatocytes of 150-day old rats from control (G9) or reduced litters (G3) fed at will (G3L), food-restricted in 30% (G3R) or refed after food restriction (G3RL).
Data shown as mean ± SD; n = 5-7 per group. [a] $p < 0.05$ *vs.* G9; [b] $p < 0.05$ *vs.* G3L; [c] $p < 0.05$ *vs.* G3R. ANOVA-Tukey.
gly: glycerol; lac: lactate; ala: alanine; gln: glutamine.

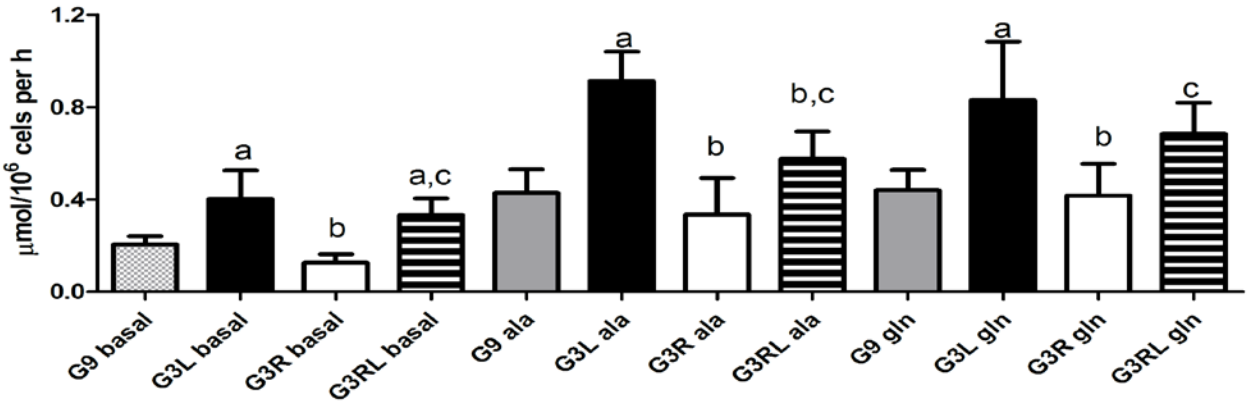

Fig. 4 Urea release by incubated hepatocytes of 150-day old rats from control (G9) or reduced litters (G3) fed at will (G3L), food-restricted in 30% (G3R) or refed after food restriction (G3RL).
Data shown as mean ± SD; n = 5-7 per group. [a] $p < 0.05$ *vs.* G9; [b] $p < 0.05$ *vs.* G3L; [c] $p < 0.05$ *vs.* G3R. ANOVA-Tukey.
ala: alanine; gln: glutamine.

the other hand, was much lower in group G3R in the presence of every precursor, while the other groups had similar gluconeogenic glucose release.

Urea release by incubated hepatocytes was determined in the presence of alanine and glutamine, as shown in Fig. 4. It was higher in group G3L than in group G9 for all three conditions, and lower in group G3R than in group G3L. Refeeding after caloric restriction (G3RL) tended to restore urea release to either G9 or G3L levels.

4. Discussion

A brief survey of the results of this work shows that 60-day old reduced-litter rats (G3L) had body weight and BMI greater than control litters (G9), but did not differ from them at later ages. Nevertheless, plasma triglycerides and VLDL were higher at 150 days of age. Food restriction of 30% from 60 to 150 days of age (group G3R) decreased body and fat weights and the lipid profile. Upon refeeding (group G3RL), these parameters were restored to those of the freely-fed (G9

and G3L) groups. In fact, visceral fat and triglycerides were higher than in group G3L.

During the insulin challenge (IIH), systemic glucose homeostasis was changed significantly in group G3R: blood glucose decay was slow, and recovery was impaired. Similarly, only this group had an overt change of glucose handling by the liver, with higher basal and lower gluconeogenic glucose release.

The effects of early life and adult feeding on hepatocyte glucose metabolism were the primary targets of this investigation. Although body weight, BMI and fat weights were not different between G9 and G3L rats at the age of 150 days, the possibility remained that litter size could have changed liver glucose metabolism. Caloric restriction increased basal and decreased gluconeogenic glucose release from incubated hepatocytes, in a fashion very similar to that seen in younger rats [9, 11]. In addition, glucose release from hepatocytes of refed animals (group G3RL) recovered the profile of rats from control or reduced litters that were fed at will since weaning (groups G9 and G3L, respectively). These two groups, in turn, did not differ significantly from each other in their basal or gluconeogenic glucose release. Therefore, litter size reduction, and the consequent early life overfeeding, did not program liver glucose metabolism in adult life. Instead, the liver seems to have a pattern of basal and gluconeogenic glucose release that is dependent on the current nutritional condition of the animal.

Increased glucose release under basal incubation conditions in the food-restricted group (G3R) suggests glucose release from endogenous stores, that is, glycogen. Although glycogen content was not measured in this investigation, the higher liver weight is an indirect indicative of this supposition. Accordingly, a previous work on younger rats under similar feeding conditions showed increased liver weight and glycogen content [11]. The higher plasma insulin levels of group G3R could have enhanced this glycogen storage, as discussed later.

Gluconeogenesis is the primary response of the liver to maintain blood glucose homeostasis, especially when glycogenolysis decreases as fasting gets longer [14-16]. The incubation of hepatocytes was made in a glucose-free medium, and the cells were obtained from overnight-fasted animals, both of which would favor gluconeogenesis, given appropriate substrates were present. Certainly, all the groups showed gluconeogenic glucose release, but the magnitude was much lower in hepatocytes from G3R rats. The supposed presence of glycogen discussed above could have decreased gluconeogenic flux in this group, or some of the early intermediates of gluconeogenesis, such as pyruvate, could have been diverted to replenish glycogen stores. This would imply that inhibition of glycogen synthesis—or stimulation of glycogen breakdown—by the counter-regulatory hormones is incomplete in the calorically-restricted rat. Alternatively, the lower urea release of group G3R in the presence of alanine or glutamine may indicate that these hepatocytes had a reduced capacity of either uptaking or metabolizing gluconeogenic precursors. Decreased urea release is also a consistent finding in this group, as it was also reported in younger G3R rats [9, 11]. In addition, lactate measurements were higher in the G3R flasks incubated with this gluconeogenic precursor (data not shown), suggesting that lactate uptake might be decreased in this group, thus leading to lower gluconeogenesis from lactate.

Glucose intolerance, assessed by the ivGTT, was not seen in any group of this investigation. However, insulin resistance, indicated by the lower kITT, was present in group G3R, and can be correlated, at least partially, with hepatocyte glucose metabolism: if glycogen stores were still present (as assumed by the high hepatocyte basal glucose release), the liver would have a decreased capacity of responding to the insulin challenge—which it would do by enhancing glucose phosphorylation and glycogen synthesis—thus delaying the glucose drop during the IIH. A similarly diminished contribution of skeletal muscle can be

speculated, as G3R animals have lower body weight, a large proportion of which is represented by this tissue.

In the food-restricted rats (group G3R), the slow and poor recovery of blood glucose during the last three hours of the IIH contrasts with the slow decay of blood glucose during the first 120 min. Exogenous insulin virtually disappears after 120 min, and counter-regulatory hormones take place to restore blood glucose to normal levels [17, 18]. Their actions are mainly liver-based, as they stimulate glycogenolysis and gluconeogenesis, thus increasing liver glucose output. As blood glucose in group G3R at time 300 min was about 50% lower than in the other groups, it seems that counter-regulatory effects on the liver were blunted. This supposition is further supported by the lower rate of gluconeogenesis in this group.

Plasma insulin did not show a consistent relation with lactational or immediate feeding conditions. First, it did not differ between groups G9 and G3L, so that lactation did not interfere with adult plasma insulin. However, this similarity in plasma insulin could be one factor why groups G9 and G3L had similar *in vivo* glucose profiles and hepatocyte glucose metabolism. Second, insulin was increased in the animals subjected to caloric restriction either prior to (G3RL) or at the time of measurement (G3R); thus, insulin was not reflecting the momentary feeding condition. Instead, the link between these groups (G3R and G3RL) and their higher plasma insulin was caloric restriction.

Certainly, the higher plasma insulin of group G3R could account for some of the metabolic changes of these rats. Higher basal insulin would decrease the buffering capacity of the liver during the insulin challenge (IIH) by continuously stimulating liver glycogen synthesis. In addition, it would inhibit gluconeogenesis [16]. These possibilities were suggested before [11]. However, *in vivo* and *in vitro* glucose metabolism in group G3RL was similar to groups G9 and G3L. Therefore, in group G3RL any effect of the hyperinsulinemia was overcome by refeeding.

In the rat colony from which the animals of this work were taken, body weight reaches a plateau by the age of 60 days. Therefore, food restriction was implemented only at this age so as not to compromise development. The 30%-caloric restriction (group G3R) decreased body weight and fat mass, and restored VLDL and triglyceride levels to those of G9 (control) animals. Although the reduced-litter rats (G3L) were not heavier or fatter than the G9 rats at the age of 150 days, they had higher VLDL, triglycerides, and atherogenic index, and the decrease of these values by caloric restriction deserves attention, as high lipid profiles and visceral fat are risk factors for cardiovascular disease and metabolic impairments [19, 20]. Unfortunately, refeeding (group G3RL) not only reversed these changes but worsened the scenario: triglycerides were even higher than in group G3L, and all visceral fats were increased. Although, statistically speaking, abdominal fats (retroperitoneal, epididymal and mesenteric) were not different between G3L and G3RL, the percentage increases (up to 24% for the mesenteric fat) cannot be regarded as biologically insignificant. In fact, a relevant role for mesenteric fat in the etiology of obesity-related metabolic syndrome was suggested [19, 20]. The same reasoning applies to the subcutaneous fat weight, which was 50% lower after refeeding (G3RL) than under continuous free feeding (G3L). These two fat deposits are very different from the metabolic point of view, with visceral fat correlating with metabolic disturbances, while subcutaneous fat is reported as having a protective role [19, 20]. Therefore, as far as fat is concerned, refeeding after caloric restriction would be increasing the metabolic risk and decreasing metabolic protection.

In summary, taking into account the proposed hypotheses of this investigation, the results indicated that the adult rat liver is not programmed or altered whatsoever by the lactational condition the animals had. On the other hand, glucose homeostasis and liver metabolism were responsive to caloric restriction *per*

se, that is to the current nutritional condition. In support of this, glucose metabolism (1) was shown to be similar between rats raised in control (G9) and reduced litters (G3L), indicating that it was not programmed by early life (lactational) feeding; (2) showed changes, both *in vivo* (ITT) and especially *in vitro* (basal and gluconeogenic liver glucose release) in reduced-litter rats under food restriction (G3R); (3) reversed to control patterns upon refeeding (G3RL).

5. Conclusions

Reducing litter size during lactation in the rat aims at mimicking human perinatal overfeeding. In both humans and rodents, this has been linked to later, adult life metabolic disturbances, a phenomenon termed metabolic programming. However, liver metabolism was largely unexplored in the reduced-litter rat, despite its central role in energy homeostasis. The results of this investigation, together with the other reports in younger rats, suggests that the liver glucose metabolism is not affected by early (perinatal) nutrition, but is responsive to the current amount of food ingested. Metabolic disturbances recorded in adult life after perinatal overnutrition possibly involve a complex interplay between organs and tissues and are related to adult adiposity. However, as long as adult weight and adiposity are kept within certain acceptable limits, the liver seems capable of maintaining glucose homeostasis. Therefore, liver glucose metabolism seems to be quite adaptable to the feeding conditions. Other changes in the intermediary metabolism, such as lipid metabolism, cannot be discarded and would deserve attention, especially considering that the major goal of human caloric restriction is to decrease adipose mass and control overweight and obesity.

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