Original article

Metabolic effects of dietary lactose in adult female rats

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Abstract — As an outgrowth of our interest in the potential toxicity of dietary galactose, we investigated the metabolic effects of high lactose diets in Long-Evans female rats. Seventy-five Long-Evans female rats (25-day-old) were randomized to receive one of 3 diets for 7 months: glucose diet (CON); low lactose diet (10.5%, LLD); or a high lactose diet (41.9%, HLD). Necropsy was performed seven months after randomization. HLD animals had significantly lower body weights than controls ($P < 0.01$). These animals continued to grow, however at a retarded rate compared to the CON group. The HLD group also had significantly lower triglyceride and non-esterified fatty acid levels than the CON group ($P < 0.01$ and $P < 0.05$). Serum glucose concentrations were lower in the HLD group compared to CON animals ($P < 0.05$), while serum insulin levels were lower than both the LLD and CON animals ($P < 0.01$ and $P < 0.05$). Leptin exhibited a similar trend. Thyroid studies revealed no difference in TSH between groups. Free T4 was significantly higher in HLD rats compared to LLD and CON rats while free T3 was lower in the HLD group ($P < 0.05$). This indicates a possible impairment in T4 to T3 conversion. Our data suggests that a long-term high lactose diet is associated with a decrease in insulin and leptin levels, and an increase in the insulin to glucose ratio. However, these changes are seen in the presence of a decreased body mass. A significant effect on thyroid hormone metabolism is also seen, and may be an adaptive mechanism in lactose-fed rats.

lactose / galactose / rat / thyroxine / thyroid metabolism

1. INTRODUCTION

Women in Western countries are urged to consume dairy products to enhance their calcium intake, with the anticipated benefit of reduction in risk of osteoporosis [1]. Since lactose is the primary carbohydrate in milk, those who consume large quantities of

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dairy products in an effort to increase calcium intake for osteoporosis prevention ingest large quantities of lactose.

In general, evidence continues to accumulate showing that macronutrients [2, 3], micronutrients [4–7] and some bioactive dietary substances [8, 9] influence metabolism and various specific systems and influence some disease risks. Concerns about potential organ or tissue-specific toxicity of dietary galactose initially grew out of the complications experienced by individuals who have galactosemia, with a number of animal studies suggesting that diets rich in galactose could produce insults that were at least somewhat comparable to those seen in galactosemics. Population-based studies have both supported and failed to support the notion that dietary intake of galactose in the form of lactose can cause target organ toxicity. Subsequent to the report by Cramer et al. in 1989 suggesting a possible link between ovarian cancer and lactose consumption [10], the relationship between lactose from milk products and ovarian cancer has been studied in greater detail [11–19]. Among these more recent epidemiological studies [13–16], results have generally not confirmed original suspicions that galactose consumption and/or slow metabolism (i.e. slow galactose transferase activity) are risk factors for ovarian cancer. A large population-based case-control study conducted in Los Angeles County, designed to test the hypothesis that galactose consumption, galactose transferase activity, and/or galactose transferase genotype were associated with ovarian cancer risk, failed to find any association [17–19].

Since the studies in the literature showing toxic effects of dietary galactose in animal models used diets rich in galactose per se and this differs from the human condition of exposure to dietary lactose, any discrepancy in outcomes might be attributable to the different form of the dietary sugar (monosaccharide versus disaccharide, etc.).

To address this possibility, we have conducted a study in female rats in which the animals were exposed to diets rich in lactose rather than galactose. Our first step in assessing the possible effects of a lactose-rich diet in this model was to measure some metabolic markers that might disclose the extent to which the disaccharide lactose might or might not differ from a reference monosaccharide, glucose.

Insulin, leptin and thyroid hormone are not only regarded as important metabolic hormones, but also, are recognized as the putative signal linking nutrition and growth, development and reproduction [20–25]. Previous studies have shown that diets containing 50–60% lactose result in diarrhea [26, 27] and dietary lactose may reduce the absorption of protein and fat [28]. Lactose also reduced plasma lipids, especially triglycerides and hepatic cholesterol accumulation in hamsters [29]. However, the effects of high lactose diets in rats on insulin, leptin and thyroid hormone are still unclear. This report describes the endocrine metabolic effects of a high lactose diet in Long-Evans rats. Specifically, the effects of a high lactose diet on anthropomorphics, insulin and leptin levels, and thyroid function are reported.

2. MATERIALS AND METHODS

2.1. Animals

Seventy-five Long-Evans female rats (25-day-old) were purchased from Harlan Sprague Dawley and maintained in air-conditioned quarters with food and water available ad libitum. The lighting schedule was 12 hour light: 12 hour dark with lights on 0500 h through 1700 h. The animals were pair-housed in the Cedars-Sinai Vivarium, which is accredited by the American Association for Laboratory Animal Care. After a washout phase on glucose, rats were

randomized to receive one of three diets: glucose diet (control), low lactose diet or high lactose diet. Necropsy was performed seven months after the start of experimental treatments. Urine was collected on the morning of necropsy. Animals were euthanized by carbon dioxide inhalation, and blood was withdrawn from the heart. The livers were collected and weighed.

2.2. Diets

To achieve relevant levels of galactose, and to present it in the form usually ingested (lactose), a semisynthetic diet that mimics the AIN 76A diet was used. Sugar intake was modified from the standard 238.6 g·1 800 cal^{-1} of sucrose. Given that the energy content of glucose and galactose are identical, glucose was used as the control. Diets were prepared by Harlan Teklad, Madison, WI. All elements of the diet remained constant throughout groups, with the exception of glucose and lactose content. In the control diet, rats were given 41.9 g glucose·100 g^{-1} of diet (CON) and no added lactose. In the low lactose diet (LLD) group, the rats received 10.5 g lactose and 31.4 g glucose·100 g^{-1} of diet. The group receiving a HLD received 41.9 g lactose·100 g^{-1} of diet (HLD) and no added glucose.

2.3. Hormone profiles

Serum insulin and leptin concentration were determined by commercial double-antibody Radioimmunoassay (RIA) kits (Linco Research Company, St. Charles, MO). The intra- and interassay coefficients of variation and the limit of sensitivity were 4.8%, 7.4% and 0.02 ng·mL^{-1}, respectively for insulin, and 3.3%, 4.8% and 0.5 ng·mL^{-1}, respectively for leptin. Serum thyroid stimulating hormone (TSH) was quantified using a immunoradiometric assay kit (Coat-A-Count TSH Diagnostic Products Corp., Los Angeles, CA) and the free T4 and free T3 were determined by commercial RIA

kits (Coat-A-Count Diagnostic Products Corp., Los Angeles, CA). The intra-assay coefficients of variation and the limit of sensitivity were 2.4% and 0.03 μIU·mL^{-1} for TSH; 5.2% and 1.29 pmol·L^{-1} for free T4; and 6.4% and 0.31 pmol·L^{-1} for free T3, respectively. Samples from all animals were analyzed together in the same assay and all analyses were performed within 2 months of sample collection.

2.4. Glucose, urea nitrogen and non-esterified fatty acids (NEFA), galactose assays

Concentrations of glucose (serum and urine), urea nitrogen and triglycerides in the serum were measured by the enzymatic methods using commercial kits (Sigma Diagnostics, St. Louis, MO). Serum concentrations of NEFA was determined by the ACS-ACOD method using a commercial kit (Wako Chemicals USA, Richmond, VA) and urinary concentrations of galactose assayed using a commercial kit (Boehringer, Mannheim, Germany).

2.5. Liver histology

Liver samples were fixed in 4% paraformaldehyde solution, transferred to 70% ethanol after 24 hours and prepared for light microscopic analysis following routine techniques. The paraffin blocks were trimmed, 5 μm sections prepared and stained with hematoxylin and eosin for light microscopic examination. Representative sections were examined at a magnification of 200×. Histopathological changes in the liver were scored on a scale with four categories: minimal; mild; moderate; or marked [30].

2.6. Statistical methods

All the data for body weight, hormone levels and metabolite concentrations are expressed as the mean standard error. The data for each end point was tested for

homogeneity of variance and treatment effects determined by One-Way ANOVA. Among- treatments effects were determined by Tukey's procedure for multiple comparisons with an alpha level of 0.05. Changes in liver histology were determined by Chi-square. All statistical procedures were performed using Sigmastat for Windows, Version 2.03 (Jandel Scientific Corporation, San Jose, CA).

3. RESULTS

3.1. Effects of lactose on urinary metabolite levels and body weight

All rats survived the experimental period without evidence of abnormal behaviors or symptoms. Clinical observations revealed no evidence of diarrhea persisting beyond the first 24 hours in the animals fed high lactose diets. As expected, a marked increase in urinary galactose excretion was seen in the HLD animals compared to CON ($P < 0.01$) and LLD ($P < 0.01$) (Tab. I) while urinary glucose levels remained constant across treatment groups. After lactose treatment for two months, the animals fed the HLD had a significantly lower body weight vs. controls (317.08 ± 6.42 vs. 294.42 ± 5.08 g, $P < 0.01$) (Fig. 1). These animals did not cease growth, however a statistically significant decrease in body mass remained a persistent finding through to necropsy (357.67 ± 9.19 vs. 316.33 ± 5.96 g, $P < 0.01$). No significant difference in weight was found between control animals and those fed a LLD. Urinary protein excretion was significantly lower in the HLD animals compared to LLD ($P < 0.05$) and CON ($P < 0.01$).
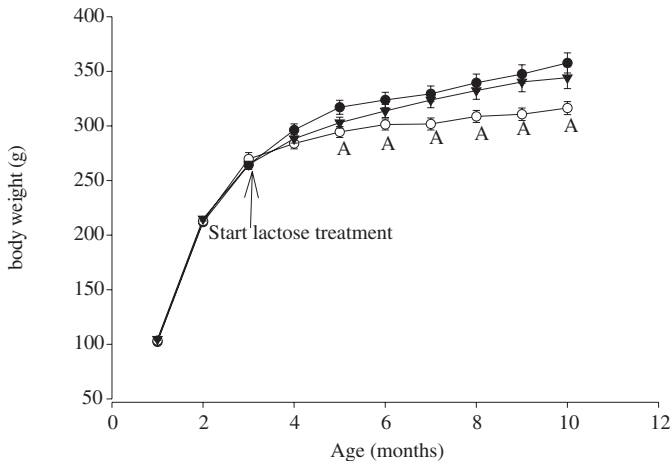

Figure 1. Effects of glucose and various levels of dietary lactose on body weight at different ages in female Long-Evans rats ($n = 75$). All rats were fed CON diet upon arrival then randomized into five groups based on their body weight. After one month, unilateral ovariectomy was performed on animals. Diets commenced one month after the ovariectomies. Each data point represents the mean $\pm$ SEM. Dietary treatment with glucose (41.9 g glucose·100 g^{-1} of diet, control, -- ● --, $n = 30$), high lactose (41.9 g lactose·100 g^{-1} of diet, HLD, -- ○ --, $n = 30$); low lactose (31.4 g lactose and 10.5 g glucose·100 g^{-1} of diet, LLD, -- ▼--, $n = 15$) began at 3 months of age. The letter "A" indicates significant differences between HLD vs. the control group $P < 0.01$.

Table I. Urinary glucose, protein and galactose concentrations

Group	N	Glucose (mg·dL^{-1})	Protein (mg·mL^{-1})	Galactose (mmol·L^{-1})
Control	30	10.71 ± 0.89	1.92 ± 0.21	0.17 ± 0.04
Low lactose	15	9.37 ± 1.96	1.66 ± 0.30	3.50 ± 0.71[A]
High Lactose	30	12.49 ± 1.10	0.93 ± 0.10[A,b]	11.11 ± 0.19[A,B]

* Indicates mean ± SEM.
[A] $P < 0.01$, compared to control.
[b] $P < 0.05$; [B] $P < 0.01$, compared to low lactose.

3.2. Effects of lactose on protein, lipid metabolism and liver histology

Serum triglyceride levels in the HLD group were significantly lower than in the control group (202.46 + 14.42 mg·dL^{-1} vs. 343.8 + 28.17 mg·dL^{-1}, $P < 0.01$) as were non-esterified fatty acid levels (1.54 + 0.12 mEq·L^{-1} vs. 1.93 + 0.11 mEq·L^{-1}, $P < 0.05$). Blood urea nitrogen levels were consistent throughout groups. The ratio of liver to body weight in rats fed a HLD was significantly higher than in rats of both the CON ($P < 0.01$) and LLD ($P < 0.01$) groups (Tab. II). Liver histology revealed no abnormalities aside from lipid accumulation. In rats fed a HLD, a minimal amount of lipid accrual was seen. Whereas in rats fed a LLD and a control diet, lipid accumulation was enhanced compared to rats fed a HLD, respectively ($P < 0.01$) (Tab. III).

3.3. Effects of lactose on serum glucose and metabolic hormones

Serum glucose concentrations were significantly lower in animals fed the high lactose diet compared to control animals ($P < 0.05$) (Tab. IV). Serum insulin levels were noted to be lower in animals receiving

Table II. Liver weights of rats at necropsy.

Items	Control	Low lactose	High lactose
Sample size	30	15	30
Body weight (g)	357.67 ± 9.19*	344.17 ± 14.06	316.33 ± 5.96
Liver fresh weight (g)	9.47 ± 0.43	9.32 ± 0.55	9.89 ± 0.27
Liver/BW (× 10^{-3})	26.38 ± 0.80	26.99 ± 1.05	31.24 ± 0.59[A,B]

* Indicates mean ± SEM.
[A] $P < 0.01$ compared to control.
[B] $P < 0.01$ compared to low lactose.

Table III. The effects of lactose on the lipid accumulation in liver.

Treatment group	Minimal*	Mild*	Moderate*	Marked*
Control[a]	15	8	4	3
Low lactose[b]	8	3	2	2
High lactose	28	2	0	0

[a] Control vs. high lactose, $P < 0.01$; [b] low lactose vs. high lactose, $P < 0.01$, by Chi-square analysis. * Number of rats.

Table IV. The effects of lactose on serum glucose, insulin, insulin/glucose and leptin.

Group	Total animals	Glucose (mg·dL^{-1})	Insulin (ng·mL^{-1})	Insulin/Glucose × 10^{-2}	Leptin (ng·mL^{-1})
Control	30	229.4 ± 9.2*	3.2 ± 0.3	1.46 ± 0.13	33.8 ± 5.3
Low lactose	15	217.6 ± 8.5	2.1 ± 0.2[A]	0.99 ± 0.12[a]	22.9 ± 5.8
High lactose	30	203.6 ± 4.8[a]	1.2 ± 0.1[A,b]	0.66 ± 0.04[A]	9.0 ± 2.1[A,b]

* Indicates mean ± SEM.
[a] $P < 0.05$; [A] $P < 0.01$, compared to control.
[b] $P < 0.05$; [B] $P < 0.01$, compared to low lactose.

Table V. Effects of lactose on thyroid function.

Group	N	TSH (μIU·mL^{-1})	Free T4 (pmol·L^{-1})	Free T3 (pmol·L^{-1})
Control	30	0.19 ± 0.03*	60.75 ± 3.73	1.11 ± 0.08
Low Lactose	15	0.24 ± 0.06	85.46 ± 6.56[B]	0.99 ± 0.12
High Lactose	30	0.21 ± 0.03	110.81 ± 4.50[A,B]	0.82 ± 0.08[a]

* Mean ± SEM.
[a] $P < 0.05$, [A] $P < 0.01$, compared to control.
[B] $P < 0.01$, compared to low lactose.

lactose diets compared to controls, with the HLD rats exhibiting the lowest serum concentrations ($P < 0.01$ compared to CON and $P < 0.05$ compared to LLD). Leptin levels exhibited a similar trend, with the HLD group having leptin levels approximately one third of the LLD group ($P < 0.05$) and one fourth of the CON group ($P < 0.01$). Thyroid studies revealed no difference in TSH values between all three groups (Tab. V). Rats fed a HLD demonstrated significantly higher free T4 levels than rats of the CON group and those fed a LLD, with free T3 values lower than controls ($P < 0.05$) indicating a possible impairment in T4 to T3 conversion.

4. DISCUSSION

Lactose is a disaccharide that is hydrolyzed by the enzyme lactase into glucose and galactose. In mammals, including humans, intestinal lactase activity is highest during the time of suckling, and declines to lower levels after weaning [31]. There are multiple reports of dietary-induced increases in intestinal lactase activity and enhanced lactose absorption with dietary manipulation [32, 33]. Rat lactase protein expression can be induced by a lactose-rich diet [34]. Based on these reports, it is apparent that lactase is an adaptive enzyme that may be induced by lactose loading in animals. Lactose-challenged rats significantly increase the expression of lactase protein by using adaptive responses and increasing their capacity to hydrolyze lactose and absorb the constituent monosaccharides [35, 36].

We observed a number of endocrine and metabolic effects of this lactose-rich diet, which mimics the predominant source of galactose in human diets. Specifically, a HLD was associated with an increase in galactose in the urine indicating that dietary lactose was absorbed and resulted in growth impairment and altered thyroid function. The absence of treatment effects on circulating levels of TSH in the face of increased levels of free T4 and suppressed free T3 are taken as evidence of a potential effect of galactose on deiodinase activity at target

tissues. We found no differences between the CON and LLD groups in the metabolic outcomes that we measured. Administration of a diet supplemented with 50% of galactose for 38 days to adults rats produced clinical symptoms such as polyuria, polydipsia and growth retardation and bilateral cataract [30]. However, in our experiment, there were no clinical symptoms and no apparent serious metabolic disturbance in the HLD and LLD groups, which suggest that there is little or no general toxicity. Our initial results suggest that ovarian toxicity is also minimal in this animal model (unpublished data).

Previous reports by other investigators have emphasized the striking effects of carbohydrate feeding on thyroid hormone metabolism in man [37, 38] and in rats [39]. In our CON-fed rats, serum free T3 levels are significantly higher than in rats fed lactose exclusive diets, which is identical to the report by Gavin et al. [40]. On the other hand, Freund et al. [41] studied the effects of thyroid hormone on lactase expression in rats. They found that injections of T4 promoted a precocious drop of enzyme activity. Interestingly, mRNA levels did not change [41]. In another study by Liu et al. [42] the turnover rate of lactase enzyme was slower in hypothyroid rats, and that lactase subunit structure was different in hypothyroid rat pups compared to euthyroid animals and those on thyroid replacement. In the hypothyroid pups, lactase structure lacked the characteristics seen in weaned pups. Thyroxine regulates lactase ontogeny by post-translational mechanisms that include altered processing and increased degradation of the lactase enzyme [42]. Hodin et al. [43] noted that T3 therapy in adult rats decreased lactase mRNA levels by approximately 75%. Therefore, it is possible that in the HLD rats, where growth depends on the ability to ingest lactose as the only carbohydrate source, a decrease in active thyroid hormone levels is adaptive. By reducing the amount of active thyroid hormone, a subsequent enhancement in lactase activity would

allow for an increased caloric intake and provide an improved anabolic environment.

Prior studies have shown that diets containing 50–60% lactose results in diarrhea [26, 27]. In this study, the amount of lactose fed was 41.9% in HLD. The lack of observed diarrhea and the continued growth support the notion of sustained lactose absorption. The increase in urinary galactose excretion adds objective credence to the clinical observations. Urinary galactose excretion is a measure of lactase activity and reflects the intestinal absorption of galactose [44, 45].

Although rats of the HLD and LLD groups continued to grow, a retarded growth rate compared to controls was seen. These results are similar to prior reports which show growth impairment in young adult rats fed diets containing > 20% lactose [46]. Previous literature on post-weaning rats has attributed this sub-optimal growth to a decrease of feed conversion [47] suggesting that dietary lactose may reduce the absorption of protein and fat.

The decrease in circulating triglyceride levels seen in rats of the HLD group may be a reflection of their overall decrease in body mass or due to reduced insulin resistance as evidenced by a decrease in the insulin to glucose ratio and lower insulin levels. Since leptin is known to correlate with body mass, the decrease in leptin levels seen in HLD rats may also be accounted for – at least in part – by the significant reduction in body mass in these animals. Hayes et al. [29] have reported that a beneficial impact is seen on lipid metabolism with increasing levels of lactose intake. Lowering of plasma lipids, particularly triglycerides, is seen in hamsters fed a diet of 30% lactose [29].

HLD rats, although leaner, were not significantly more steatotic. In fact, they stored less fat in their liver than animals from the other 2 groups. Liver accumulation of fat is a common finding in starvation [47]. The lack of this observation supports the theory that these animals, although lean, are not

cachetic or starving. The higher ratio of liver to body weight in this group of animals is directly related to the decrease in weight as opposed to an increase in liver mass. Conversely, the accumulation of lipid in the livers of the glucose-fed animals suggests a relationship between their increased body weight and expected lipid accrual.

Serum glucose concentrations were significantly lower in the HLD group, as were overall insulin levels resulting in a decrease in insulin to glucose ratios in rats fed a HLD. The decrease in absolute insulin levels and the reduction in the insulin to glucose ratio can be accounted for in part by the weight difference among the three groups of rats. In addition the lower levels of non-esterified fatty acids in these animals provide an environment for enhanced insulin sensitivity [48]. Interestingly, Wijayashinge et al. [49] showed that calves fed high lactose low fat milk diets developed hyperglycemia and glucosuria while Hugi et al. [50, 51] noted that male calves receiving a high lactose intake had a reduction in insulin utilization during the latter stages of growth. It is possible that the weight reduction seen in our HLD rats effectively negated any propensity to develop insulin resistance. It is possible that the changes in insulin profiles secondary to the ingestion of high amounts of lactose differ in ruminant and non-ruminant animals. There was a significant increase in blood glucose and in plasma insulin only 60 min after 25 g lactose ingestion in type 2 diabetic patients [52, 53]. Although diabetes is associated with increased intestinal lactase activity in humans [54], we do not know of any reports that assess the long-term effects of lactose rich diets on serum glucose and insulin in humans.

In Long-Evans rats, the effect of weight loss complicates the evidence for systemic hormonal effects of a high lactose diet. In particular, changes outlined in lipid profiles, glucose and insulin homeostasis and leptin levels may all be attributable to the weight reduction seen during lactose feeding. Our observations of thyroid hormone changes in rats on high lactose diets warrant further investigation. It is possible that these changes are adaptive mechanisms allowing for enhanced nutrient absorption and utilization. The precise relationship between lactose ingestion, and thyroid hormone activity remain elusive. Further work is needed to define the role of lactose on deiodinase activity, and to elucidate the mechanisms of thyroid hormone influence on lactase activity.

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