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Insulin Resistance Induced by Early Life Overnutrition is Reversed by Calorie Restriction

Leila S. Joseph, Saleh Mahmood and Mulchand S. Patel

Department of Biochemistry, Jacob School of Medicine and Biomedical Sciences, University at Buffalo

Abstract

Overnourishment of the rat during the suckling period results in the development of hyperinsulinemia, adult-onset obesity and insulin resistance. To investigate insulin resistance in the skeletal muscle of adult male rats, the litter size was reduced from 12 (NL) to 3 (SL) male pups/dam from postnatal day 3 to day 21. Both NL and SL rats were fed lab chow *ad libitum* until day 140. Another SL group was pair-fed (SL/PF) to NL rats starting from day 21. On day 94, one half of the SL/PF rats continued to be pair-fed, while the remaining SL/PF rats were allowed *ad libitum* feeding of chow (SL/PF/AL). Insulin receptor substrate-1 (IRS-1) levels were significantly reduced in skeletal muscle of SL rats but pair-feeding normalized IRS-1 levels in SL/PF rats. However switching to *ad libitum* feeding maintained IRS-1 levels similar to that of NL. These results show that pair-feeding was adequate to reverse insulin resistance in SL/PF rats.

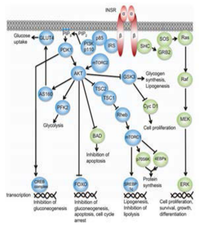
Introduction

Obesity is a large concern from both public and economic standpoints today, especially in Western Societies. Statistics show that 35.7% of the adult population in the U.S. alone is classified as obese (w/ BMI's > 30), which is an increase from previous time periods. The presence of obesity puts people who have it at higher risk for several metabolic disorders such as hypertension, adverse lipid concentrations and type II diabetes. Even though genetics, sedentary life style, and increased consumption of high-caloric foods are the major causes underlying the current obesity epidemic, epidemiological data and results from animal models suggest that altered nutritional experiences during early periods in life (i.e. fetal and suckling period) can potentiate development of obesity and metabolic diseases later in the offspring's life. The maturation of organs such as the pancreas and the hypothalamus neuronal system in rodents is completed only in the immediate postnatal period, so it is during this period that malnutrition can affect developmental programming. While these early adaptations enable the organism to endure the nutritional stress, in the long run they are disadvantageous. It has been demonstrated that cross-fostering of normal rat offspring by a diabetic dam resulted in metabolic disorders in adulthood.

A rat model for metabolic programming effects due to changes in nourishment during the suckling period is the small liter (SL) mode in which the litter size is reduced to 3 pups/dam. Overnourishment induced by the reduction of litter size for newborn pups resulted in increased levels of serum insulin and leptin and increased body weight gains during the suckling period. Insulin resistance also resulted. It has been shown that deviations in insulin-stimulated glucose uptake can play a major role in the pathogenesis of insulin resistance. This condition is proven to be associated with alterations in the components of the insulin signaling cascade including reduced serine phosphorylation of the insulin receptor and IRS (insulin receptor substrate) proteins. These effects persisted in the postweaning period with the manifestation of obesity and other metabolic disorders in adulthood.

Introduction (cont.)

It has been shown that caloric restriction can reduce body weight gain and improve health circumstances of obese models. Since the obese phenotype in SL pups was observed during the suckling period and persisted in the postweaning period, it was hypothesized that caloric restriction from the time of weaning may have a positive impact of the development of obesity in adult life via the reversal of early programming effects. So to investigate this, we pair-fed (PF) SL rats to NL rats starting on day 21 followed by the effects of *ad libitum* feeding on day 94. Our results indicate that pair-feeding was able to normalize the levels of protein content of involved in the insulin signaling pathway, although not permanently since *ad libitum* feeding reduced those levels.



Insulin Signaling Pathway

Methods

SL model. Pregnant rats were obtained from Charles River laboratories (Wilmington, MA). On postnatal day 3, pups were assigned randomly to normal litter (NL) or small litter (SL). NL group litter size was adjusted to 12 male pups/ dam to provide similar level of nourishment, while SL group was adjusted to 3 male pups/dam for overnourishment. SL pups were nursed by their natural dams, while NL pups were randomly assigned to dams. For studies on adult animals, male pups were weaned on d21 and were housed individually. NL and SL rats had *ad libitum* access to lab chow and water.

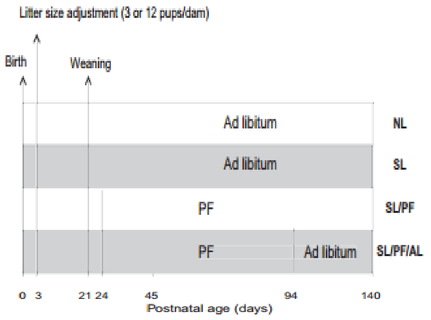
Body weight and food intake. To determine the immediate effects of overnourishment due to litter size reduction, body weights of NL and SL pups were measured from d3 to d24. For adult rats, body weight and food intake were measured on a weekly basis for all groups.

Mild CR (pair-feeding regimen, equalizing food intake to that of NL rats). The pair-feeding regiment was initiated from d24 and continued until d140. The daily food supply based on quantity of rodent chow consumed by NL rats was given to SL/PF rats. On d94, ½ of the SL/PF rats continued being pair-fed while remaining SL/PF rats were allowed *ad libitum* feeding and are referred to as SL/PF/AL rats.

Tissue collection. On d140, all groups of rats were anesthetized by intraperitoneal injection of ketamine and xylazine followed by decapitation between 9:30 and 11:30AM. Region including gastrocnemius and soleus muscles was dissected out and immediately frozen in liquid nitrogen and stored at -80^{o} C.

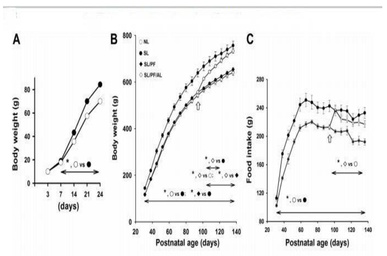
Methods (cont.)

Western blotting. Skeletal muscle samples were homogenized in ice-cold lysis buffer and supernatant was obtained by centrifugation. Total protein in supernatant was measured by BCA method (Bio-Rad, Hercules, CA, USA). Proteins were separated using SDS-PAGE and detected using specified antibodies.

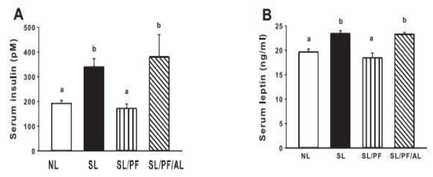


Experimental groups are depicted schematically. SL, small liter; NL, normal litter; PF, pair feeding; AL, *ad libitum* feeding.

Previous Findings

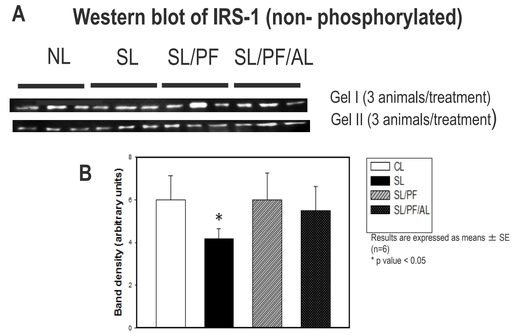


Body weights of NL and SL rats from postnatal day 3 (d3) to d24 (A); NL: n 26; SL: n 34 PF (left). Body weights (B; n 9 – 12/group) and mean weekly food intake (C; n 9 –12/group) of NL, SL, SL/PF, and SL/PF/AL rats from d28 to d140. Results are expressed as means ± SE.

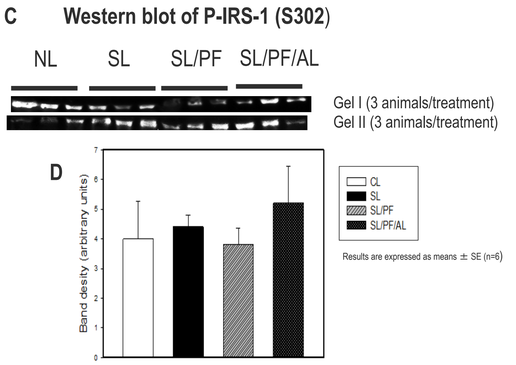


PF serum insulin (A) and leptin (B) levels for NL, SL, SL/PF, and SL/PF/AL. Results are expressed as means ± SE; n = 8-9 group/ group. Statistical analysis was performed using ANOVA followed by post hoc analysis using Student-Newman-Keuls Method. Significance ($P < 0.05$) among groups is denoted by different letters (a, b, c).

Results



Results: IRS-1 levels in skeletal muscle were significantly reduced in SL rats compared to NL rats. Pair-feeding of SL/PF rats resulted in normalization of levels to those of NL rats. However switching to *ad libitum* feeding (SL/PF/AL) did not have any effect on the level of IRS-1. These results show that there is less expression of IRS-1 of SL/PF rats to SL rats, implying that there is an alteration in the insulin signaling pathway. Pair-feeding appears ameliorate the impairment.



Results: There was no significant change in the protein level of serine phosphorylated IRS-1 between NL and SL rats. Pair-feeding (SL/PF group) and *ad libitum* feeding (SL/PF/AL group) also had no effect. These results suggest that the developmental programming induced by overnutrition of SL rats has no effect on phosphorylation of a specific serine residue (S302) of IRS-1. Phosphorylation of the S302 residue in IRS-1 results in inhibition of its action.

Summary

- Overnutrition of rats in the postnatal period leads to the reduction of the levels of IRS-1 in skeletal muscle, indicating a defect in the insulin-signaling pathway. This may contribute to the development of insulin resistance reported in SL rats.
- Pair-feeding normalized IRS-1 levels in skeletal muscle in SL rats, and was sufficient in reversing the developmental programming effects brought by overnutrition in the postnatal period.
- Serine (S302) phosphorylation of IRS-1 is not affected by overnutrition during the postnatal period. This is an odd finding in view of insulin resistance in SL rats.