

# The haematological profile with fifty generations of undernutrition cannot be reversed with six generations of a recuperation diet in a wistar rat colony

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## Abstract

David Barker and colleagues in Southampton, England were trying to identify all-cause mortality and morbidity in the English population. They found out that undernutrition during foetal life was primarily responsible for the adult morbidity and mortality. These adaptations or “Programming” were necessary for survival of the foetus in an unfavourable environment. Poor health of the mother during pregnancy was responsible for the increased risk of cardiovascular disease, stroke and diabetes in these offspring. This led to the articulation of “The Foetal Origins of Adult Disease” hypothesis which states that, “Undernutrition in foetal life acts to program the risk for early onset of disease in the adult life”. Similar studies carried out in India have revealed the “Thin-Fat” Indian phenotype. These initial observations have been confirmed later by many human and animal studies. We have previously reported that Wistar Rats (Thrifty Jerry) that have been undernourished for fifty generations show leukopenia, macrocytic anaemia and increased platelet count. The present study tried to evaluate if a standard rat diet (Recuperation diet) provided could reverse the harmful effects of 50 generations of undernutrition. The blood indices and the platelet count were restored after 6 generations of a recuperation diet. However, the white blood cell count was higher along with the presence of lymphocytosis and granulocytosis. 6 generations of the recuperation diet could not completely reverse the effects of multigenerational (50 generations) undernutrition.

**Key Words:** Foetal Programming, Multigenerational Undernutrition, Complete Blood Count, Recuperation Diet

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## INTRODUCTION

David Barker’s epidemiological studies in Southampton, England showed that under-nutrition in foetal life leads to

adaptations that can be detrimental in adult life. His thus pioneered the concept of “Foetal Origins of Adult Disease” (Godfrey and Barker 2000). This work has been substantiated and expanded by both animal as well as many human studies. These adaptations or “programming” are a result of the changes that are at a hormonal, cellular as well as metabolic level (McMillan and Robinson 2005). Yajnik *et al* have studied these adaptations in the Indian population and have found that Indians at birth are centrally obese, insulin resistant but have a lower soft tissue mass, “The Thin-Fat Phenotype” (Yajnik, Fall *et al* 2003). As a result of these adaptations, coronary heart disease, hypertension and Diabetes Mellitus in developing countries like India is very common and has assumed epidemic proportions

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(Echouffo-Tcheugui 2012). We have studied the effects of multigenerational undernutrition in a Wistar rat model (Thrifty Jerry) and have shown that these rats mimic the Indian phenotype. These animals are centrally obese, show insulin resistance, deficiency of Vitamin B<sub>12</sub> and folate levels along with lower soft tissue growth (Hardikar *et al* 2015). Studies carried out on the hematopoietic environment in undernourished animals have shown the presence of hypocellularity, necrosis, and extracellular matrix modifications (Travlos GS (2006), Fried W *et al* (1978), Vituri CL(2000), Prestes-Carneiro *et al* (2006) and Borelli P *et al*(1995). The Thrifty Jerry rats showed the presence of leukopenia, macrocytic anaemia and increased platelet count with fifty generations of undernutrition (M.M.Karandikar *et al*). The present study evaluated the role of providing a standard rat diet (recuperation diet) to the undernourished animals (transition colony) for 6 generations.

## MATERIALS AND METHODS

The undernourished wistar rat colony was given a standard rat pellet diet for 6 generations- “Transition Colony”. 10-week-old adult female rats from the 6<sup>th</sup> generation of the transition colony were studied. The control group received a standard rat feed. Blood was collected by retro-orbital bleeding and serum was immediately separated and analysed for Complete Blood Count (CBC) on an auto analyser (Mindray BC2800). The study was ethically approved by the institutional ethics committee.

### Statistical Analysis

All estimations were carried out in triplicates and values are expressed as +/- Std Error of Mean (SEM). The statistical significance was evaluated by the unpaired t test using Graph Pad Prism 6 version software.

## RESULTS

**Table 1: Complete Blood Counts in Control and Transition Adult Wistar Rats**

Estimations	Control Mean ± SEM	Recuperation Mean ± SEM	P value
HCT	30.57 ± 5.535, n= 6	38.80 ± 1.011, n= 5	0.2160
MCV	53.68 ± 0.6635, n= 6	53.56 ± 0.6875, n= 5	0.9007
MCHC	29.58 ± 0.3458, n= 6	30.40 ± 0.1000, n= 5	0.0674
RDW****	14.97 ± 0.3685, n= 6	11.34 ± 0.4045, n= 5	< 0.0001
PLT	812.3 ± 40.98, n= 6	706.4 ± 46.48, n= 5	0.1204
MPV	7.067 ± 0.5506, n= 6	5.067 ± 1.018, n= 5	0.1148
PDW	15.53 ± 0.2092, n= 6	14.90 ± 0.08944, n= 5	0.0295
PCT	0.4960 ± 0.05083, n= 6	0.3694 ± 0.07310, n= 5	0.1781
WBC****	5.700 ± 0.7933, n= 6	8.940 ± 0.4589, n= 5	0.0087
Lymphocytes****	81.38 ± 2.022, n= 6	56.02 ± 0.8261, n= 5	< 0.0001
Monocytes**	2.783 ± 0.2926, n= 6	3.900 ± 0.2121, n= 5	0.0157
Granulocytes****	15.87 ± 1.808, n= 6	40.08 ± 0.9661, n= 5	< 0.0001
RBCs	5.648 ± 1.001, n= 6	7.258 ± 0.1753, n= 5	0.1838
Hb	9.017 ± 1.656, n= 6	11.68 ± 0.3338, n= 5	0.1851

Significance: \* p<0.01, \*\* p<0.001, \*\*\*/\*P<0.0001

## DISCUSSION

The foetal origins of adult hypothesis proposed by David Barker and his colleagues analysed the role of foetal undernutrition and diseases in adult life. His studies have shown that foetal undernutrition cause hypertension, coronary heart disease and type 2 diabetes in adult life. (Godfrey and Barker 2000). These adaptations labelled as “programming” are due to adjustments in a variety of systems that occur during fetal life and occur at the structural, metabolic and hormonal level. Since David Barker’s studies, human and animal studies have confirmed and modified these initial observations and is now called as Developmental Origins of Adult Disease (DoHAD) (McMillan and Robinson 2005). The Pune Maternal Nutrition Studies (PMNS) by Yajnik *et al* have

studied the Indian scenario that have led to the understanding of “Thin-Fat” Indian phenotype [Yajnik, Fall *et al* (2003), Yajnik and Deshmukh, (2012)]. We have carried out studies in a Wistar rat model (Thrifty Jerry) that has been undernourished for 50 generations. The animals were insulin resistant, centrally obese and had smaller visceral organs (Hardikar *et al*). Studies carried out so far have primarily focused on the endocrine, metabolic, cardiovascular and renal adaptations to undernutrition. A few studies have evaluated the role of undernutrition on the haemopoietic environment and have shown the presence of Hypocellularity, necrosis and extracellular matrix modifications (Travlos GS (2006), Fried W *et al* (1978), Vituri CL (2000), Prestes-Carneiro *et al* (2006) and

Borelli P *et al* (1995). However very few studies have evaluated the role of multigenerational undernutrition (50 generations). We have previously reported the role of multigenerational undernutrition on the blood cell counts and have shown leukopenia, macrocytic anaemia and increased platelet count in these animals (M.M. Karandikar *et al*). This study has evaluated the role of providing a standard rat diet (Recuperation diet) to the undernourished animals for 6 generations. The transition colony showed the presence of leucocytosis (5.70 in Control vs 8.94 in Transition  $\times 10^3/\mu\text{L}$ ). This was associated with granulocytosis (15.87 vs 40.08 %) and lymphopenia (81.38 vs 56.02%). This was suggestive of a state of acute infection. This exact cause is unknown and would require further evaluation. The macrocytic anaemia as seen in the undernourished colony was completely corrected with 6 generations of a recuperation diet. MCV (53.68 vs 53.56  $\mu\text{m}^3$ ) and MCHC (29.58 vs 30.40 g/dl) was normalized. Red cell count, hematocrit and hemoglobin levels were also normal in the transition colony. This showed that 6 generations of a recuperation diet could completely reverse the macrocytic anaemia.

Hardikar *et al* have evaluated the same effect in 2 generations of a recuperation diet. They could show partial improvement but the changes were not completely reversed. 6 generations of the recuperation diet were required to reverse the changes of macrocytic anemia. Interestingly red cell distribution width showed a significantly lower value (14.97 vs 11.34) showing cells that had no anisocytosis or poikilocytosis. This needs further evaluation. This study also showed that platelet levels were normalized in the transition colony after 6 generations of the recuperation diet. We have not carried out bone marrow studies nor have we looked the epigenetic changes that could be involved in this transition colony. Further studies that would involve both bone marrow evaluation and epigenetic studies could throw light on the adaptations and reversal of the macrocytic anaemia and restoration of the platelet count. The presence of granulocytosis is indicative of an acute state of infection in these animals. This study has thus shown that 6 generations of a normalised diet could not completely reverse the effects of 50 generations of undernutrition in the Thrifty Jerry Colony.

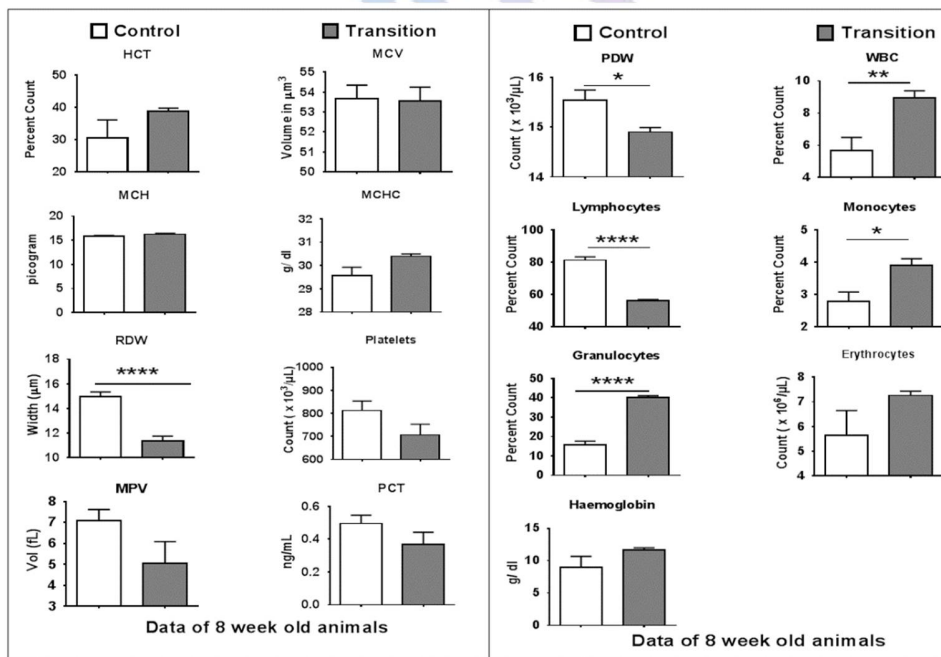


Figure 1: Blood Cell Count in Control and Transition Adult Female Wistar Rat

Figure 2: Blood Cell Count in Control and Transition Adult Female Wistar Rat

## CONCLUSIONS

Multigenerational undernutrition for more than 50 generations that causes macrocytic anaemia can be reversed with 6 generations of a recuperation diet. This is however associated with leucopenia, granulocytosis and lymphopenia indicative of an inflammatory state.

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