

# Maternal Caloric Restriction prior to Pregnancy Increases the Body Weight of the Second-Generation Male Offspring and Shortens Their Longevity in Rats

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Maternal undernutrition can affect offspring's physical status and various health parameters that might be transmittable across several generations. Many studies have focused on undernutrition throughout pregnancy, whereas maternal undernutrition prior to pregnancy is not sufficiently studied. The objective of our study was to explore the effects of food restriction prior to and during pregnancy on body weight and longevity of the second generation offspring. Adult female Wistar rats ("F0" generation) were 50% food restricted for one month prior to pregnancy (pre-pregnancy) or during pre-pregnancy and pregnancy. The third group was fed normally (control). The first generation offspring were normally fed until the 6<sup>th</sup> month of age to produce the second generation offspring; namely, the first-generation female rats were mated with male breeders from outside the experiment. The second generation offspring thus obtained were observed until natural death (up to 36 months). Compared to the controls, the second-generation male offspring whose "grandmothers (F0 females)" undernourished only during pre-pregnancy were significantly heavier from the 8<sup>th</sup> month of age, whereas no significant weight difference was found in the male offspring whose "grandmothers" were food-restricted during pre-pregnancy and pregnancy. Shorter lifespan was observed in the second-generation male offspring of "grandmothers" that were food-restricted either during pre-pregnancy or during pre-pregnancy and pregnancy. By contrast, no differences in body weight and lifespan were observed in all second-generation female offspring. In conclusion, maternal caloric restriction prior to pregnancy increases the body weight and shortens the longevity of the second-generation male offspring, indicating the sex-dependent transgenerational effect of maternal caloric restriction.

**Keywords:** offspring; pregnancy; pre-pregnancy; rat; undernutrition

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## Introduction

Obesity, traditionally attributable to high welfare countries, has not bypassed the poorer regions recently (Hoffman et al. 2000; Yajnik 2004; Varela-Silva et al. 2007; Hult et al. 2010; Myles et al. 2011; Bhuiyan et al. 2013). In addition, modern culture is excessively propagandized with an extra slim body image that can also be responsible for the development of metabolic diseases (Yu et al. 2013). Since metabolic changes leading to obesity and associated chronic diseases manifest in various environments as well as at a young age, they can no longer be explained solely by sedentary lifestyle or an overconsumption of high-energy-dense food. Following historical evidence (post-war com-

munities, Dutch hunger winter, others) a theory explaining obesity and cardiovascular diseases pandemic by fetal life events emerged (Barker and Martyn 1992; Yajnik 2004; Kyle and Pichard 2006; Koupil et al. 2007). Studies describe a wide range of possible metabolic alterations as a result of an insufficient diet during pregnancy. These include small birth weight (Frederick et al. 2008; Pinheiro et al. 2008; Yu et al. 2013), delayed maturation (van Weissenbruch et al. 2005; Guzman et al. 2006), altered endocrine and nervous regulation of metabolism (McArdle et al. 2006; Sellayah et al. 2008; Coupe et al. 2010; Myles et al. 2011; Peixoto-Silva et al. 2011; Reusens et al. 2011a, b; de Oliveira et al. 2012; Garcia et al. 2013), obesity (Hoffman et al. 2000; Kinra et al. 2000; Ozanne and Hales

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2004; Yajnik 2004; Varela-Silva et al. 2007), inadequate immune response (Beach et al. 1982; Reynolds et al. 2013), greater risk of non-communicable diseases in adulthood (Benyshek et al. 2006; Kyle and Pichard 2006; Koupil et al. 2007; Torrens et al. 2008; Harrison and Langley-Evans 2009; Hult et al. 2010; Norman et al. 2012; Ponzio et al. 2012) as well as changes in cognitive function (Blaise et al. 2007; Gilbert et al. 2010; Zhang et al. 2010; Ito et al. 2011) or even circadian rhythms (Orozco-Solis et al. 2011). However, this subject is rarely examined through a lifetime, and only a glimpse of possible consequences is traced. There are a number of studies examining the effects of undernutrition in the first generation offspring; however, only a few deal with changes of subsequent offspring generations (Stewart et al. 1975; Beach et al. 1982; Benyshek et al. 2004, 2006; Zambrano et al. 2005; Pinheiro et al. 2008; Harrison and Langley-Evans 2009; Peixoto-Silva et al. 2011; Ponzio et al. 2012). Thus, there is a particular need in research in the context of long-term outcomes such as aging, longevity and especially its inheritance to future generations. Furthermore, most studies concentrate on undernutrition solely during pregnancy, failing to examine the role of the period of formation of maternal metabolic capital, the pre-pregnancy period, that can be of crucial importance for the development of the fetus (Benyshek 2007; Wells 2010).

The objective of this study was to explore the impact of maternal nutrition before and during pregnancy on body weight of the offspring (F1 and F2) as well as the development of chronic diseases and risk of premature death of second generation offspring (F2).

## Methods

The animal husbandry and experiments on animals were carried out according to the National and European regulations and were approved by the National Animal Care and Use Committee (Permission No. 0211).

The total 12 mature female Wistar rats (F0 generation) were divided into 3 groups in respect to nutritional restriction (Fig. 1). The rats were fed either normal diet ("Control group") or restricted diet: one experimental group was food restricted for one month prior to and during pregnancy ("Pre-pregnancy and pregnancy food restricted group") and the other, one month prior to pregnancy only ("Pre-pregnancy food restricted group"). Food restricted rat groups received 50 percent less of the feed eaten in the control group. After birth, no food deprivation was applied and all offspring (F1 generation) were fed according to the recommended daily intakes. First generation offspring (F1;  $N = 35$ ) was observed until the 6<sup>th</sup> month of age, and then F1 females were mated with the proven male breeders from outside the experiment to produce the second generation offspring (F2). The F1 males were euthanized after 6 months of age, whereas the F0 and F1 female rats were euthanized after weaning of the offspring. The second generation offspring (F2;  $N = 87$ ;  $n = 43$

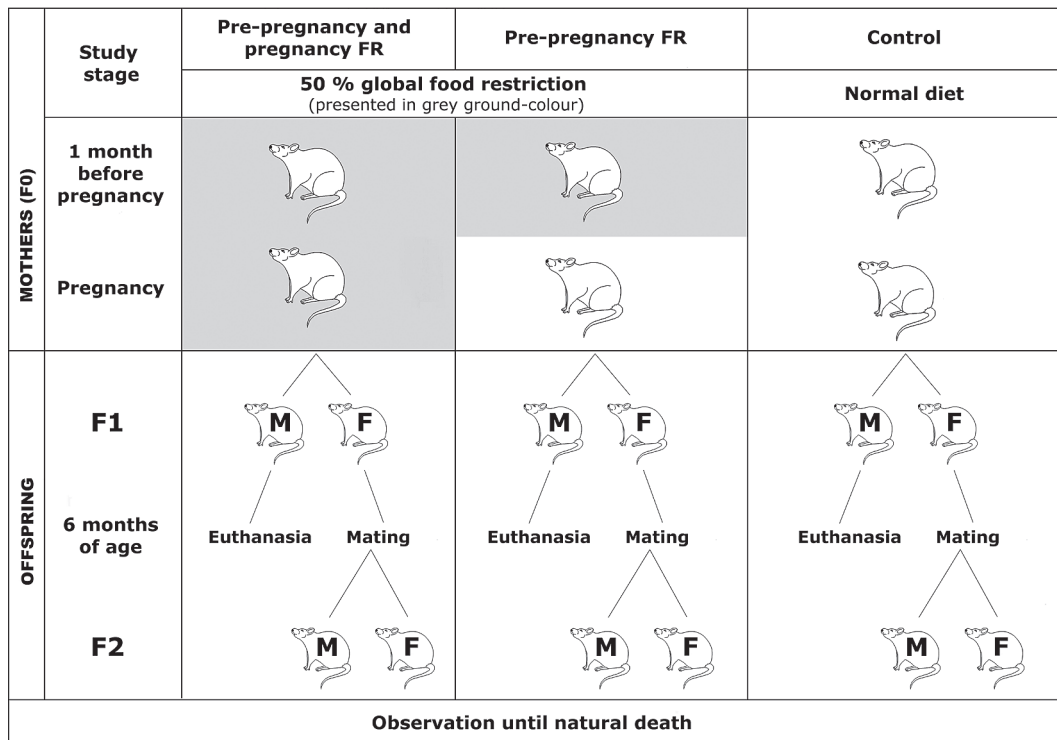


Fig. 1. The design of the study.

F1, first generation offspring; F2, second generation offspring; M, males; F, females. F0 maternal rats consumed either normal diet (Control group) or were food restricted before pregnancy (Pre-pregnancy FR group), or before pregnancy and in utero (Pre-pregnancy and pregnancy FR group). The F0 and F1 females were mated with the proven male breeders outside of the study and euthanized after weaning of the offspring. The F2 offspring were observed until natural death.

females,  $n = 44$  males) were kept and weighed under the same standardized conditions until natural death (up to 36 months). Body weight was measured weekly with calibrated scales starting at one month of age. However, body weight graphs were composed for 32 months as only few animals survived longer thereby graphic weight visualisation and statistical analysis were no longer appropriate.

Data analysis was performed using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY). The data were expressed as z-scores (for improved graphic visualisation and interpretation z-scores were chosen as the distance between an individual rat's weight and the average weight of the investigated rats) and tested for normality using Shapiro-Wilk normality test. Weight differences among the groups were compared using the one-way analysis of variance (ANOVA) followed by Bonferroni *post-hoc* test; on a few occasions where data was not normally distributed, weight differences were compared using Kruskal-Wallis one-way analysis of variance. Furthermore, mixed design ANOVA was used to analyze the body weight data over time. Survivability analysis was performed using the Kaplan-Meier estimate followed by the Tarone-Ware test. Censored values were those few ( $n = 5$ ) randomly removed from the control group in the first year of the study for the histopathological comparison (to be published elsewhere) as well as survivors. The value of  $p$  less than 0.05 was considered statistically significant.

## Results

The mixed design ANOVA revealed that there was a significant effect of maternal undernutrition on the growth of the second-generation offspring rats ( $p = 0.041$ ) in respect to gender and the period of food restriction. The analysis revealed statistically significant differences among food-restricted groups ( $p = 0.03$ ) as well as between sexes ( $p < 0.001$ ) in the second generation offspring. The statistically significant weight differences among the second-generation offspring groups were observed until approximately the 22<sup>nd</sup> month of age when a reduction in sample affected the reliability of the analysis. The mixed design analysis of variance did not reveal statistically significant effect of maternal undernutrition on the overall growth of the first

generation offspring ( $p = 0.168$ ) (the latter result might be attributable to the small sample size).

The one-way ANOVA did not find any weight-related differences among all first-generation (F1) female offspring groups (experimental, i.e. food restricted, and controls) during the first 6 months of life (Fig. 2). Moreover, there were no differences observed in body weight of the second-generation (F2) female offspring rats for almost an entire 3-year study period. Female offspring rats whose “grandmothers” were food-restricted before pregnancy (“Pre-pregnancy food restricted group”) were lighter than those of the control group only at 1 month of age ( $p = 0.02$ ) (Fig. 3).

The one-way ANOVA revealed significantly bigger weight of the first-generation (F1) male offspring whose mothers were food restricted prior to and during pregnancy (“Pre-pregnancy and pregnancy food restricted group”) in comparison to the other offspring groups since the 5<sup>th</sup> month of age (Fig. 4). Compared to the controls, no significant weight differences were found in the second-generation (F2) male offspring whose “grandmothers” were food restricted prior to and during pregnancy (“Pre-pregnancy and pregnancy food-restricted group”) (Fig. 5). The latter offspring group had heavier weight than the control offspring group in the 1<sup>st</sup> and 2<sup>nd</sup> month of life. However, body weight of F2 adult male offspring whose “grandmothers” were food-restricted before pregnancy (“Pre-pregnancy food-restricted group”) was significantly bigger than that of the control group starting from the 8<sup>th</sup> up to the 22<sup>nd</sup> month of life with a minor exception in the 15<sup>th</sup> month. Besides, the aforementioned offspring group had bigger weight in comparison to F2 male offspring rats whose “grandmothers” were food-restricted before and during pregnancy (“Pre-pregnancy and pregnancy food restricted group”) in the 12<sup>th</sup> month, also during the 16-21<sup>st</sup> month of life (Fig. 5).

Survival functions differed by gender and experimental group (for females: Fig. 6 and Table 1; for males: Fig. 7 and Table 2). The second-generation male offspring of all

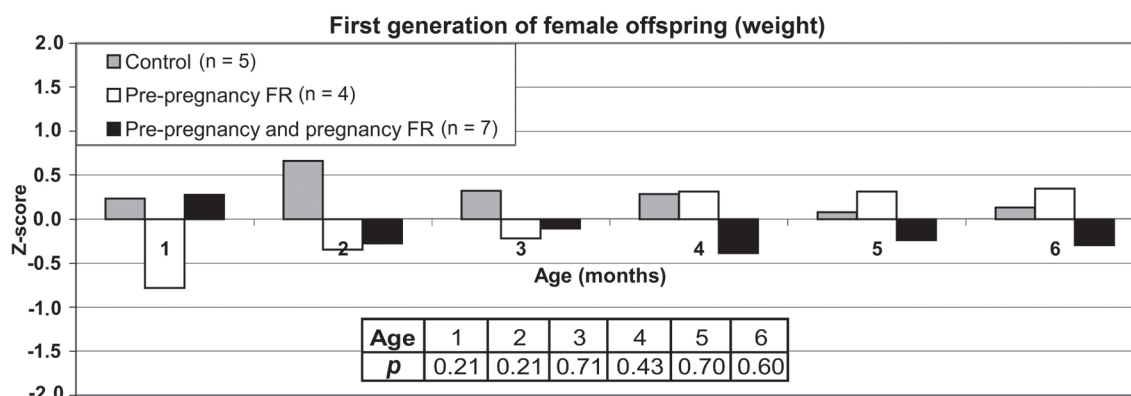


Fig. 2. Weight dynamics in the first generation of female offspring rats.

FR, food restricted. Data show *z-score* means in weight for three experimental rat groups of the first-generation female offspring (F1). F0 maternal rats consumed either normal diet (Control group) or were food restricted before pregnancy (Pre-pregnancy FR group), or before pregnancy and in utero (Pre-pregnancy and pregnancy FR group). The table inside of the figure shows the  $p$  values obtained from ANOVA analysis of variance comparing body weight among F1 female groups monthly.

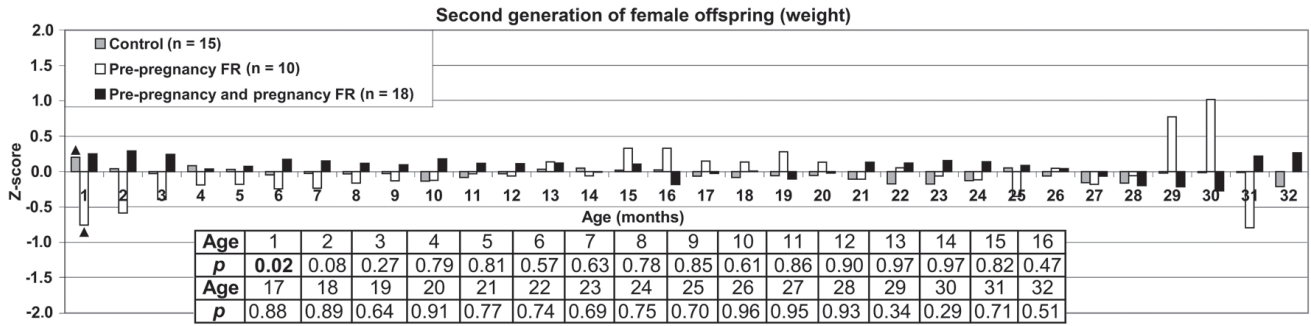


Fig. 3. Weight dynamics in the second generation of female offspring rats.

FR, food restricted. Data show *z-score* means in weight for three experimental rat groups of the second-generation female offspring (F2). F0 maternal rats consumed either normal diet (Control group) or were food restricted before pregnancy (Pre-pregnancy FR group), or before pregnancy and in utero (Pre-pregnancy and pregnancy FR group). ▲ *p* < 0.05 control group *versus* pre-pregnancy FR group. The table inside of the figure shows the *p* values obtained from ANOVA analysis of variance comparing body weight among F2 female groups monthly.

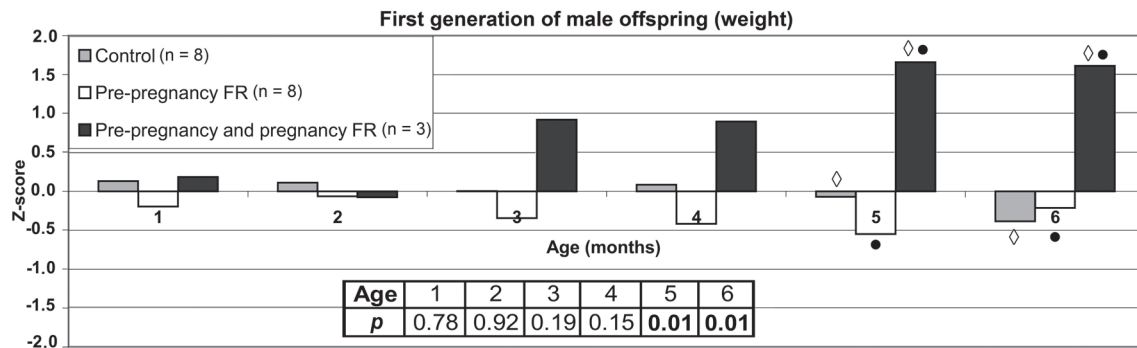


Fig. 4. Weight dynamics in the first generation of male offspring rats.

FR, food restricted. Data show *z-score* means in weight for three experimental rat groups of the first-generation male offspring (F1). F0 maternal rats consumed either normal diet (Control group) or were food restricted before pregnancy (Pre-pregnancy FR group), or before pregnancy and in utero (Pre-pregnancy and pregnancy FR group). ◇ *p* < 0.05 control group *versus* pre-pregnancy and pregnancy FR group; ● *p* < 0.05 pre-pregnancy FR group *versus* pre-pregnancy and pregnancy FR group. The table inside of the figure shows the *p* values obtained from ANOVA analysis of variance comparing body weight among F1 male groups monthly.

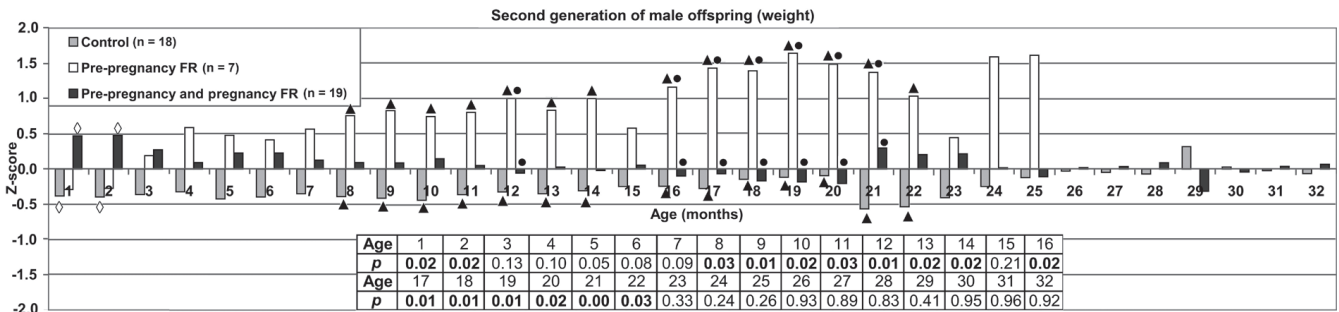


Fig. 5. Weight dynamics in the second generation of male offspring rats.

FR, food restricted. Data show *z-score* means in weight for three experimental rat groups of the second-generation male offspring (F2). F0 maternal rats consumed either normal diet (Control group) or were food restricted before pregnancy (Pre-pregnancy FR group), or before pregnancy and in utero (Pre-pregnancy and pregnancy FR group). ▲ *p* < 0.05 control group *versus* pre-pregnancy FR group; ◇ *p* < 0.05 control group *versus* pre-pregnancy and pregnancy FR group; ● *p* < 0.05 pre-pregnancy FR group *versus* pre-pregnancy and pregnancy FR group. The table inside of the figure shows the *p* values obtained from ANOVA analysis of variance comparing body weight among F2 male groups monthly.

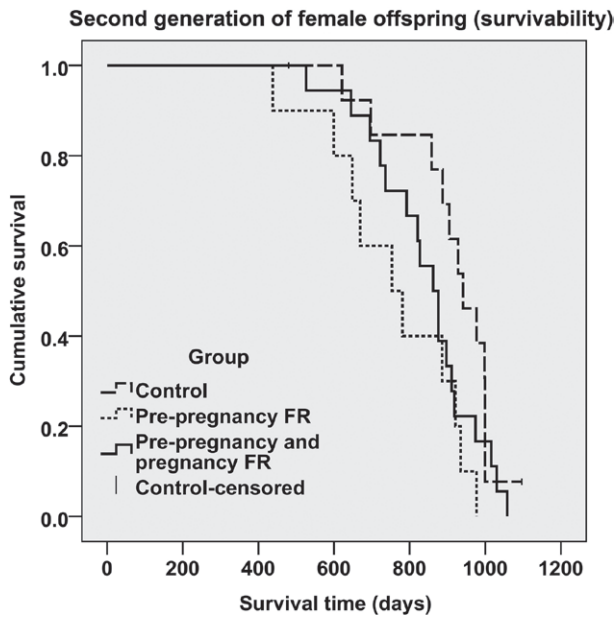


Fig. 6. Survivability of the second generation of female offspring rats. FR, food restricted. Data show survivability parameters for three experimental rat groups of the second-generation female offspring (F2). F0 maternal rats consumed either normal diet (Control group) or were food restricted before pregnancy (Pre-pregnancy FR group), or before pregnancy and in utero (Pre-pregnancy and pregnancy FR group).

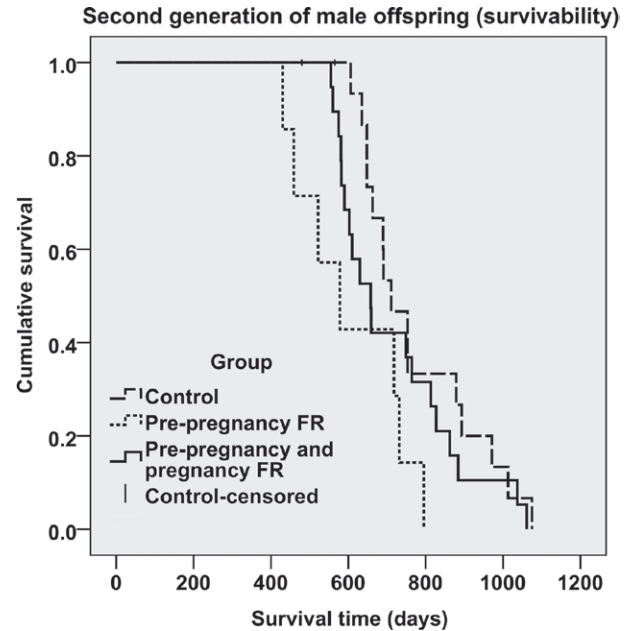


Fig. 7. Survivability of the second generation of male offspring rats. FR, food restricted. Data show survivability parameters for three experimental rat groups of the second-generation male offspring (F2). F0 maternal rats consumed either normal diet (Control group) or were food restricted before pregnancy (Pre-pregnancy FR group), or before pregnancy and in utero (Pre-pregnancy and pregnancy FR group).

Table 1. Survivability statistics of the second generation of female offspring rats.

| Investigated group             | Mean                 |            |                         |             | Median               |            |                         |             |
|--------------------------------|----------------------|------------|-------------------------|-------------|----------------------|------------|-------------------------|-------------|
|                                | Survival Time (days) | Std. Error | 95% Confidence Interval |             | Survival Time (days) | Std. Error | 95% Confidence Interval |             |
|                                |                      |            | Lower Bound             | Upper Bound |                      |            | Lower Bound             | Upper Bound |
| Control                        | 915.8                | 34.7       | 847.7                   | 983.8       | 941.0                | 43.1       | 856.4                   | 1,025.6     |
| Pre-pregnancy FR               | 760.7                | 54.8       | 653.3                   | 868.1       | 753.0                | 88.5       | 579.5                   | 926.5       |
| Pre-pregnancy and pregnancy FR | 841.9                | 31.6       | 779.9                   | 903.8       | 862.0                | 34.6       | 794.1                   | 929.9       |
| All groups combined            | 849.2                | 24.1       | 801.9                   | 896.5       | 886.0                | 22.4       | 842.1                   | 929.9       |

FR, food restricted. Data show survivability parameters for three experimental rat groups of the second-generation female offspring (F2). F0 maternal rats consumed either normal diet (Control group) or were food restricted before pregnancy (Pre-pregnancy FR group), or before pregnancy and in utero (Pre-pregnancy and pregnancy FR group).

Table 2. Survivability statistics of the second generation of male offspring rats.

| Investigated group             | Mean                 |            |                         |             | Median               |            |                         |             |
|--------------------------------|----------------------|------------|-------------------------|-------------|----------------------|------------|-------------------------|-------------|
|                                | Survival Time (days) | Std. Error | 95% Confidence Interval |             | Survival Time (days) | Std. Error | 95% Confidence Interval |             |
|                                |                      |            | Lower Bound             | Upper Bound |                      |            | Lower Bound             | Upper Bound |
| Control                        | 775.3                | 39.2       | 698.5                   | 852.0       | 711.0                | 30.4       | 651.4                   | 770.6       |
| Pre-pregnancy FR               | 604.9                | 54.5       | 498.1                   | 711.6       | 578.0                | 73.3       | 434.3                   | 721.7       |
| Pre-pregnancy and pregnancy FR | 714.8                | 35.1       | 646.0                   | 783.6       | 658.0                | 35.5       | 588.3                   | 727.7       |
| All groups combined            | 721.2                | 25.5       | 671.3                   | 771.1       | 690.0                | 33.7       | 623.9                   | 756.1       |

FR, food restricted. Data show survivability parameters for three experimental rat groups of the second-generation male offspring (F2). F0 maternal rats consumed either normal diet (Control group) or were food restricted before pregnancy (Pre-pregnancy FR group), or before pregnancy and in utero (Pre-pregnancy and pregnancy FR group).

food restricted “grandmothers” died earlier than the control offspring ( $p = 0.044$ ). Female offspring survivability did not differ among groups ( $p = 0.052$ ).

### Discussion

Experimental and epidemiological studies demonstrated a range of metabolic adaptations to ensure conception and childbearing in both human and non-human populations (Dufour and Sauther 2002). Prenatal nutrition remains crucially important for subsequent growth, and even mild changes in caloric intake can influence maternal as well as the offspring metabolism. Both underweight and overweight before pregnancy can cause similar problems such as inadequate birth weight and subsequent chronic diseases later in life (Benyshek 2007; Pinheiro et al. 2008; Yu et al. 2013). There are a number of studies assessing biometric, hormonal and metabolic changes in several periods of development: pregnancy and lactation. However, the importance of the maternal metabolic capital – the physical status during pre-pregnancy seems to be underestimated and poorly explored. In addition, most studies are carrying “express research”, capturing the effects of early undernutrition on the first generation offspring only, and for a brief period of time. Hence, the data on the consequences of the developmental programming in the context of aging and transmittance to future generations is almost non-existent.

The present study has concentrated on body weight and longevity of second generation offspring, as first generation offspring are relatively well described. Moreover, the first-generation (F1) offspring body weight data gained at the present study supported the worldwide data. Generally, offspring born to food restricted mothers are prone to obesity, and this tendency is usually observed in males rather than females (Bieswal et al. 2006; Pinheiro et al. 2008; Bol et al. 2009). In contrast to the first-generation offspring studies, the second-generation studies rarely find dramatic differences in body weight between prenatally food restricted and control groups. This may be attributable to the short observation time (ranging from 70 days to up to 1 year (Benyshek et al. 2004; Zambrano et al. 2005; Pinheiro et al. 2008; Peixoto-Silva et al. 2011) and the fact that most studies researched solely protein restriction rather than global nutrient restriction.

Studies find either no difference in offspring birth weight (Zambrano et al. 2005; Pinheiro et al. 2008) or a low birth weight (Benyshek et al. 2004, 2006; Peixoto-Silva et al. 2011) of the first generation offspring and no changes in body weight at birth or even macrosomia (Benyshek et al. 2004; Pinheiro et al. 2008) in the second generation offspring. Apart from the described early consequences on biometric parameters of the offspring, maternal undernutrition can also lead to hypoxia of the foetal brain (Ito et al. 2012). In agreement with previous reports, the present study has not found any weight differences in adult female offspring throughout all the study period (Benyshek et al. 2004; Zambrano et al. 2005; Pinheiro et al. 2008; Peixoto-

Silva et al. 2011). Female offspring seem to be more adaptive and alleviate some of the damage caused by early undernutrition in previous generations. Female offspring born to “grandmothers” that starved during pregnancy and pre-pregnancy differed from the control group at 1 month of age only, then “caught up” and continued to grow normally. However, this is not always the case for the male offspring. Studies show that both generations can develop changes in body mass, insulin sensitivity alterations, adipocyte hypertrophy, cardiovascular defects as well as leptin resistance later in life (Zambrano et al. 2005; Benyshek et al. 2006; Pinheiro et al. 2008; Harrison and Langley-Evans 2009; Peixoto-Silva et al. 2011; Ponzio et al. 2012). The present study also complements findings concerning no weight difference between pregnancy food restricted offspring and the control rats in adulthood in the second generation offspring (Benyshek et al. 2004; Zambrano et al. 2005; Peixoto-Silva et al. 2011). However, others find that male second generation offspring are heavier and longer than the controls at 6 months of age (Pinheiro et al. 2008).

One of the unifying results in transgenerational nutrition research is the consequence of malnutrition during the lactation period. Studies agree that lactation is a crucially important period of development where undernourished offspring can acquire a range of components of metabolic syndrome and some damage caused by undernutrition during gestation can be restored (Ozanne and Hales 2004; Benyshek et al. 2004, 2006; Pinheiro et al. 2008; Peixoto-Silva et al. 2011). Other studies argue that moderate protein restriction in the early stages of life (during the lactation period) does not cause obesity or related diseases, but is responsible for underweight and hypoinsulinemia (Zambrano et al. 2005; Gravena et al. 2007; de Oliveira et al. 2012). Notwithstanding, with severe restriction, diabetogenic tendencies persist (Pinheiro et al. 2008).

The majority of other authors did not study nutrition before pregnancy and its outlying effects on biometric parameters of the second generation offspring. The present study found that the second-generation male offspring born to pre-pregnancy food deprived “grandmothers” were significantly heavier than those in other groups. We agree to previous hypotheses that pre-pregnancy undernutrition might exhaust mother’s metabolic potential and impair adequate nutritional environment for the offspring (Wells 2003, 2010). Apparently, mother’s body size adjusts to an energy saving regiment as an adaptation for an expected scarce resources environment (Gluckman et al. 2005; McArdle et al. 2006). Nevertheless, these conditions change in time of pregnancy since no nutritional deprivation is applied. We presume that mother’s organism might be “re-programmed” to live in a nutritionally prosperous environment with a tendency to store energy. Hence, even in the second-generation male offspring are heavier than those born to the control group.

The present study has not observed the dynamics of biochemical indices of chronic diseases. Yet, other second-

generation studies find that even in the case of normal body size, offspring born to undernourished mothers exhibit quite dramatic endocrine and cardiovascular alterations. The second-generation offspring face increased insulin concentration (Zambrano et al. 2005; Pinheiro et al. 2008) as well as insulin resistance, altered glucose metabolism (Benyshek et al. 2006; Pinheiro et al. 2008; Peixoto-Silva et al. 2011), and increased concentrations of cholesterol and triacylglycerols (TAGs) (Peixoto-Silva et al. 2011). Studies also observe adipocyte hypertrophy and an increase in leptin concentration by more than 2 times in the first generation offspring and 6 times in the second generation offspring (Peixoto-Silva et al. 2011) signalling severe leptin resistance. Cardiovascular studies reported intergenerational transmission of elevated blood pressure as well as an endothelial dysfunction and reduced nephron number for up to three generations of offspring (Benyshek et al. 2006; Harrison and Langley-Evans 2009; Ponzio et al. 2012). In addition, the restriction of micronutrients such as zinc can negatively affect the immune function in three generations of offspring (Beach et al. 1982).

A range of visible indices of metabolic stress in the second generation offspring of food restricted “grandmothers” was also observed (Araminaite et al. 2013). Red tears (chromodacryorrhea), neck and face oedema, change in grooming practices, hair loss and others are considered important markers of rodent stress as well as health risks (Harkness and Ridgway 1980; Moyaho and Valencia 2002; Zhou et al. 2013). Behavioural alterations such as fearfulness, or contrarily, poor reaction to stimuli are described in various studies as a likely consequence of prenatal undernutrition (Gluckman et al. 2005; Zhang et al. 2010). Human studies even consider major affective disorder to be a result of prenatal undernutrition (Brown et al. 2000). However, future studies are needed.

The present study has found that pregnancy or pre-pregnancy undernourished rat dams’ male offspring have a reduced life expectancy compared to those of the control group. The comparable data are extremely scarce. Other first generation research complement our findings that animals undernourished in utero experience catch up growth in later life (Ozanne and Hales 2004; Aerts and Van Assche 2006; Pinheiro et al. 2008; Peixoto-Silva et al. 2011; Norman et al. 2012) and die younger than the control animals, in particular, overfed individuals – their life expectancy can be reduced by as much as 50% (Ozanne and Hales 2004). In contrast, those fed normally in utero, but exposed to protein restriction postnatally, have an increased longevity (Ozanne and Hales 2004).

It was hypothesized that aforementioned metabolic alterations tend to normalize in subsequent generations (Drake et al. 2005), however, not all studies corroborate these findings. The first generation offspring might have no evident endocrine changes; however, the second generation offspring can inherit impaired development of the pancreas, and have high glucose and insulin levels leading to later

insulin resistance (Benyshek et al. 2004; Pinheiro et al. 2008; Peixoto-Silva et al. 2011). In addition, second generation offspring have higher serum TAGs and leptin levels than first generation offspring (Peixoto-Silva et al. 2011). Conflicting results demonstrate a further need for high quality transgenerational investigation.

The exact underlying mechanisms behind prenatal undernutrition are still under discussion. Authors attribute these changes to an imbalanced autonomous nervous system caused by early undernutrition (de Oliveira et al. 2012). Studies suggest that protein restriction in utero might alter formation of the hypothalamus and thus “malprogramme” fetal development (Sellayah et al. 2008; Coupe et al. 2010; Peixoto-Silva et al. 2011). It is also estimated that foetal undernutrition causes mitochondrial dysfunction (Reusens et al. 2011b), as well as telomere shortening (Tarry-Adkins et al. 2009) in the pancreas resulting in lower energy synthesis and an inadequate antioxidant balance which might consequently lead to metabolic syndrome. Recent studies have linked changes in the skeletal as well as cardiac muscle composition with later cardiovascular pathogenesis (Tarry-Adkins et al. 2013; da Silva Aragao et al. 2014). The above changes demonstrate that early nutrition might have a dramatic impact on all organ systems at a cellular as well as the subcellular level.

Metabolic programming “likes” consistency – maternal and fetal nutritional environment should be like that of the postnatal nutritional environment (Ozanne and Hales 2004; Sellayah et al. 2008). Some studies have suggested the compensatory effects of prenatal undernutrition with adequate postnatal nutrition or hormonal therapy (Reynolds et al. 2013; Watez et al. 2014), whereas other authors remain quite pessimistic. Animal research shows that a spare diet from a young age might be helpful in preventing age induced damage on pancreatic  $\beta$  cells (He et al. 2012). Unfortunately, there is still little knowledge on the ideal nutritional conditions following prenatal undernutrition, but it seems that high-energy postnatal diets are not among the best choices (Ozanne and Hales 2004; Benyshek et al. 2004; Chaabo et al. 2010). Others argue that maternal nutrition before pregnancy should be a key period for interventions (Wells 2003) since an inadequate maternal capital might still induce an energy saving mechanism responsible for future obesity and risk of premature death, as the present study showed. The present study has demonstrated that pregnancy as well as the pre-pregnancy environment influences the development of the offspring of two generations. Current knowledge of the epigenetic changes leading to later chronic diseases is primarily based on short-term animal research and retrospective human studies, and thus further studies of growth programming patterns are still needed.

The present study revealed that the consequences of maternal undernutrition should be examined further on the macroscopic, endocrine, metabolic and cellular levels of multiple generations of offspring. Holistic research strate-

gies might be essential to identify the exact interaction among the prenatal adaptation, aging and general health status.

In conclusion, maternal nutritional restriction before or during pregnancy alters not only the body weight but also the longevity of the second generation offspring. The consequences are evident in males, especially those born to female offspring of pre-pregnancy food-deprived mothers. This study shows the necessity for further intergenerational research of the phenotypic traits in respect to metabolic, physiologic and epigenetic changes at the first and second generation offspring.

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### Conflict of Interest

The authors declare no conflict of interest.

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