Divergent Physical Activity and Novel Alternative Responses to High Fat Feeding in Polygenic Fat and Lean Mice

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Received: 29 November 2007 / Accepted: 27 February 2008 © Springer Science+Business Media, LLC 2008

Abstract We determined whether altered physical activity levels might underlie the contrasting adiposity of a divergently selected polygenic murine model of metabolic syndrome (Fat; F) and leanness (Lean; L) mice. We measured physical activity with a long term running wheel experiment and performed an additional high fat diet intervention. Further, we measured posture allocation by visual monitoring within the home cage as a non-exercise correlate of ‘normal’ physical activity. Whilst initially similar, running wheel activity of the F line declined with age, while the activity of the L line increased. Food intake was higher in the L line and increased with wheel exposure. Vertical rearing measured by video quantification in the home cage, without the stimulus of a running wheel was also significantly higher in the L line. The two lines developed novel alternate strategies to defend their body weight when exposed to high fat diets with a running wheel. F mice increased their running wheel activity, and despite unaltered food intake, still gained weight. L mice reduced their food intake and maintained activity levels without a significant change in body weight. Phenotypic selection for divergence in body fat content has co-segregated with a genetic predisposition for divergent physical activity levels and different strategies for coping with exposure to high fat diets that will facilitate the discovery of the genes underlying these important obesity related traits.

Keywords Obesity · Physical activity · Running wheels · Fat percentage · Metabolic syndrome

Introduction

Obesity is a major risk factor for several serious disorders including type II diabetes, coronary heart disease and hypertension. As such, the economic impact of the obesity ‘epidemic’ is enormous, with costs running into billions of dollars annually in the US alone (Frost 2005; Li et al. 2005; Wolf and Colditz 1998). Obesity is widely acknowledged to stem from an interaction between environmental and genetic factors (Bunger and Hill 2005; Mutch and Clement 2006; Speakman 2004; WHO 1998). Knowledge of the genetic basis of variations in body composition may therefore contribute to efforts to alleviate the condition.

It is widely acknowledged that obesity results from a sustained mismatch between energy intake and expenditure. In mammals energy expenditure comprises three main components: resting metabolic rate (RMR), thermoregulatory demands and physical activity (PA). In humans thermoregulatory demands are trivial whereas energy
expended on physical activity comprises approximately 30% of total energy expenditure (Goran and Treuth 2001). There is evidence that low levels of physical activity in today’s western society could be an important factor leading to the development of obesity (Tsai et al. 2003; Weisner et al. 1998; Waller et al. 2007). Direct measures of physical activity in obese subjects indicate that they are less physically active than their lean counterparts (Ekelund et al. 2005; Levine et al. 1999), although this may result from, rather than cause the obesity. Recent evidence has suggested that exercise-independent habitual allocation of posture to more energy demanding positions (Non-Exercise Activity Thermogenesis; NEAT—standing, fidgeting, etc.) is biologically determined and may also influence weight gain (Levine et al. 1999, 2005). However, in contrast, several longitudinal studies have indicated no association between variation in the energy expended on physical activity and subsequent weight gain (Tataranni et al. 2003; Wareham et al. 2005).

The polygenic Fat (F) and Lean (L) mice were selectively bred to model complex polygenic human obesity, by divergent selection on body fat percentage (%fat, from generation 20 onwards) from an initial cross between two inbred (CBA, JU) and one outbred (CFLP) strains (Sharp et al. 1984). The body fatness increased in the F line from 10% to 22% and decreased to 4% in the L line (Bunger and Hill 1999). Energy budget analysis of the F and L mice (Bunger et al. 2003) indicated that the two lines had similar food intake, suggesting F line obesity does not result from hyperphagia, unlike the polygenic New Zealand Obese mice (Jurgens et al. 2006). Indeed, subsequent high fat feeding studies indicated that the L line actually consumed more energy (Morton et al. 2005) suggesting that differences between the F and L lines reside in the energy devoted to physical activity or thermoregulation costs (Bunger et al. 2003).

To address the hypothesis that increased physical activity accounts for the divergent fatness of the lines we measured F and L activity with running wheels (exercise activity) as well as video-based posture allocation (NEAT-like) behaviour. Furthermore, we exposed F and L mice to high fat diets to determine if the exaggerated divergence in adiposity under these conditions (Morton et al. 2005) may have a basis in altered physical activity.

Materials and methods

The long term selection and further development of the F and L lines and details of the genetic basis of the line divergence and the inbreeding period are described elsewhere (Bunger and Hill 1999; Horvat et al. 2000; Stylianou et al. 2004; Stylianou et al. 2005). Mice derived from the inbred lines were used in this study (Bunger and Hill 1999). Extensive characterisation of our lines in previous studies (Bunger and Hill 1999; Bunger et al. 2003) determined that body weight gain is highly correlated with fat mass accretion in fat mice. This was substantiated in several genetic mapping experiments (Horvat et al. 2000; Stylianou et al. 2004) demonstrating that the fat% trait and body weight trait co-localise to the same QTL regions with significant LOD scores. We followed bodyweight change closely and interpreted the changes in our lines as being primarily due to altered body fat mass.

Running wheel experiment 1: Male mice (L, n = 15, F, n = 7) were studied starting at approximately 7 weeks of age. Mice were first acclimated for 3 days in cages with wheels. For experiment 1 the wheel had a diameter of 15 cm and distance per revolution was 0.47 m. Mice were maintained on a standard 16 h: 8 h light dark photoperiod (lights on at 5 am) at the institution. Running wheel experiment 2: Male L and F mice (n = 6 per line) at 23–26 weeks of age were similarly acclimated to cages with running wheels. For experiment 2 the wheel had a 25 cm diameter and distance per revolution was 0.78 m. In this experiment, performed in an independent laboratory, the light dark cycle was 12 h: 12 h (lights on at 7 am), the standard light/dark conditions for the institution. Please note that despite differences in the light dark cycle of the animal facilities, similar results between the lines were obtained (see Results). For determination of posture allocation, a video camera was used to record 10 mice of each line individually in clear standard home cages. Mice (n = 10) were acclimated to single caging for 2 weeks prior to testing to avoid interference of novel environment stress on their behavior. This allowed us to then quantitate the pronounced rearing behavior we had noticed in the L mice during their light phase and further distinguished home cage physical activity from feeding bouts (found predominantly during the dark phase). In order to formally quantitate the pronounced repetitive jumping behavior we had noticed in the L mice, we ran 5 min trials on each mouse during the light phase (between 10 am–12 noon). The videos were analysed manually and the number of vertical movements, average time of each individual rearing event and total time spent in vertical movements within the 5 min period, such as rearing, and repetitive jumping, was recorded.

Animals were fed on pelleted Rat and Mouse breeder and grower diet (Special Diets Services, SDS, UK Ltd., Witham, Essex, UK) or with defined low (11% calories as fat with sucrose; D12329) and high fat (58% calories as fat with sucrose; D12331) diets (Research Diets, New Brunswick, New Jersey). Differences between the lines (Fig. 1a, b) were analysed by RM ANOVA followed by a post hoc General Linear Model procedure of the SAS System for Windows release 9.1.3 (SAS Institute Inc., Cary, NC).
27513, USA) on an XP-PRO platform and by regression analyses using the R programme (R Development core team 2005). P-values below 0.05 were accepted as statistically significant. Effects of line and diet on food intake and running wheel activity were also analysed by 2-way RM ANOVA, followed by post-hoc Holm-Sidak multiple comparison tests using Sigmastat version 3.5 (Systat Software). The difference between baseline running activity (Fig. 1e) and weight gain on high fat diet (Fig. 3c) were analysed by 1 way ANOVA with post hoc Holm-Sidak testing (Sigmastat). All animal experiments were performed according to local ethical guidelines and within the Scientific Procedure Act (1986) of the UK Government Home Office.

Results

L mice have increased wheel running activity

Experiment 1: The mean running wheel activity data (RWA, average no. of wheel revolutions per 24 h) during the 42 days of measurements and food intake data for the period of 24 days are presented in Fig. 1a. The average RWA in F mice (5651 ± 185.4 Rv/d) was significantly lower than L mice (11106 ± 126.1 Rv/d); effects of line \(F = 591.67, P < 0.001\), time \(F = 2.48, P < 0.001\) and the interaction \(F = 2.01, P < 0.005\) were all significant. Levels of running were initially similar, but diverged with age; line differences were statistically significant on day 11...


(P < 0.05) and from days 16–42 (P < 0.05). The RWA of the F mice progressively decreased from around 7,000 (3.3 km per day) to 4,000 revolutions/day (~1.9 km), which was about 40% of the L-mice RWA. In contrast, the L mice increased activity from around 7,000 to more than 10,000 revolutions per day (~4.7 km) (Fig. 1a). Both lines exhibited a similar linear trend in weight gain despite their very different levels of RWA.

The weight gain during the running wheel trial was significant, on average 5 g (P = 1.8E–08) from their initial weight of 23 g and 6 g (P = 0.002) from their initial weight 30 g for the L and F mice, respectively.

Daily food consumption on regular chow diet was initially similar and diverged with age (significantly different between the lines during days 6–30 of the running wheel trial; Fig. 1a). The average food intake in the F mice (4.93 ± 1.065 g/d) was significantly lower than in the L mice (6.36 ± 1.427 g/d), effects of line (F = 345.4, P < 0.001), time (F = 3.24, P < 0.001) and the interaction (line by time [F = 2.1, P = 0.0019]) were all significant. Overall, the L line mice ate on average 1.4 g more food per day than the F mice in this period (P = 3.3E–15). Daily food intake increased with increasing RWA in both lines (Fig. 1b), but the slope of the regression was higher in the L (0.001 g/rev.) than in the F line (4E–05 g/rev., P < 0.05) suggesting that the L mice ate more on days when they ran more. Running in both lines occurred predominantly during the active dark phase of the cycle, the L mice ran for longer periods during the dark phase (Fig. 1c, d).

Recent characterisation of the largest effect QTL for fatness in the F line revealed that the adiposity is determined in part by early- and late-onset mechanisms (Stylianou et al. 2004). Experiment 1 used younger animals that, whilst beyond the linear part of their growth curve (up to approximately 9 weeks), were still growing. We also performed running wheel tests on older (23–26 weeks, n = 6) L and F mice which is informative concerning divergent responses at the age-onset end of the obesity equation and is perhaps of increased relevance to human obesity and metabolic syndrome (Stylianou et al. 2004). Experiment 2 was performed in an independent laboratory where the standard light/dark cycle is 12 h: 12 h (Fig. 1d). We noted that L mice had already established increased food intake at this age as we described previously (Morton et al. 2005). Higher total running counts were recorded for both lines in this experiment compared to the younger mice (Fig. 1e), likely due to both the longer dark period and because the mice were considerably older (for reference, running wheels were 0.78 m/revolution). L mice (n = 6) ran significantly more (Fig. 1d, lower actogram; y-axis spikes indicate intensity, or number of revolutions/time bin of the running bouts) than F mice (Fig. 1d, upper actogram). L mice had increased total daily activity (Fig. 1e, revolutions per day [F = 8.552, P = 0.017]) compared to F mice and, as for young mice (Fig. 1c) this was accounted for by a longer duration of running in the active dark phase.

L mice have increased vertical posture allocation

We also noticed that L mice were more active than F mice when in their home cages during their light phase, exhibiting a pronounced rearing and jumping behaviour. We therefore quantitatively examined the behaviour of L and F mice by video in their home cage during the light phase as a better indicator of a ‘normal’ physical activity, independent of the dark phase running wheel experiments. Monitoring of vertical posture allocation revealed both the number of vertical movements (Fig. 2) (F = 8.221, P = 0.0102) and total time spent vertical (L: 58 ± 3 s vs. F: 35 ± 6 s [F = 9.986, P = 0.005]) was significantly higher in L mice. The average time spent in individual rearing events was not significantly different between the lines (L: 2.4 ± 0.1 s vs. F: 2.3 ± 0.2 s, P = 0.7).

L and F mice adopt alternative strategies on exposure to high fat diet

We have previously demonstrated that high fat feeding exaggerates the divergence in weight gain in the F and L mice [21]. That is, L mice lose fat mass and F mice gain fat mass with excess calories despite the higher average caloric intake in the L line (Morton et al. 2005). Since our data showed that physical activity might contribute to the divergence of adiposity, we wished to examine if high fat feeding would also invoke a divergence in this behaviour.
that might account for the exaggerated divergence in fat mass with high fat feeding (Morton et al. 2005). F and L mice \((n = 6)\) were allowed to acclimate to the running wheel and given a defined control diet that has a high carbohydrate (sucrose) and low fat content. We monitored wheel running activity and food intake and observed as before that L mice ate more calories, (Morton et al. 2005), and ran more than F mice (Fig. 3a). However, when we switched the F and L mice to a high fat diet \((5.56 \text{kcal/g})\) we noticed that L mice significantly reduced their calorie intake (Fig. 3b, line by diet interaction \([F = 10.421, P = 0.009]\), with a highly significant effect of diet in the L mice, \(P < 0.001\) and maintained their running rate with a tendency for body weight loss (Fig. 3c). There was no recorded food wastage and previous experience with these diets does not indicate L mice have an aversive response to the highly palatable high fat diets (Morton et al. 2005 and data not shown). In contrast, in response to high fat diet, F mice increased their running rate (increased total counts Fig. 3a, line by diet interaction \([F = 16.281, P = 0.002]\), with a highly significant effect of diet in the F mice, \(P < 0.001\)) and maintained a similar calorie intake (Fig. 3b). As well as increasing total running counts, F mice also increased their running intensity (peak counts per minute) in response to high fat feeding significant interaction between line and diet \((F = 48.046, P < 0.001)\) peak counts were: F control diet: 83 ± 3 cpm, F high fat diet: 127 ± 6 cpm, highly significant effect of diet \(P < 0.001\) in F mice, compared to L control diet: 90 ± 3, L high fat: 107 ± 11, no effect of diet within L line, \(P = 0.117\).

Despite this, F mice significantly increased their body weight with high fat feeding (Fig. 3c, significant difference between lines \([F = 6.208, P = 0.034]\)) suggesting that energy efficiency (calorie storage vs. expenditure) in F mice was increased, via other mechanisms, when they were challenged with a high fat diet.

**Discussion**

Previously, energy budgets were analysed for the F and L mice (Bunger et al. 2003). Total daily energy assimilation and RMR measurements allowed calculation of the combined energy expended on physical activity and thermoregulation. There was a major difference between the F and L lines in this component of the energy budget, but it was not possible to dissect the contribution of physical activity to the divergent energy expenditure. The main objective of the present study was therefore to test the hypothesis that activity levels account for the divergent adiposity. Wheel running data in chow fed young mice initially indicated no significant difference in activity. However, after 16 days of exposure to the wheels, young L line mice began to run more than the F line. The F mice steadily decreased activity throughout this period. Our data confirm the hypothesis generated from earlier indirect measurements (Bunger et al. 2003) that the L mice were physically more active than the F mice. Notably, although not directly measured, there was no evidence of altered thermogenesis between the lines in these earlier experiments (Bunger et al. 2003). Future direct studies on fuel utilisation, using metabolic chambers would be necessary in order to answer this question, in particular with regards to the unexplained effects showing increased weight gain despite increased activity with high fat feeding in the F mice. The earlier energy budget calculation experiments
(Bunger et al. 2003) were performed in home cages, without running wheels and our current data on posture allocation show that L mice were also more active without running wheel stimulus. Independent analysis of running wheel activity on older mice housed under a different light cycle showed again a clear and highly significant increase in running wheel activity in the L line mice.

The quantity of food intake of the F and L line in the initial part of this study (on day 6) was similar, and comparable to the food intake observed in the previous study when energy budgets were quantified in cages without running wheels (Bunger et al. 2003). The food consumed by young F and L mice did not differ initially, but was increased in the L line from day 6 onwards in the long term running wheel experiment 1. The increase in food intake of the L line paralleled their increased physical activity. In addition, the strong relationship between the daily food intake variation and running wheel activity suggested that the L line increased their food consumption in response to the prolonged increase in physical activity. Consistent with this, mice selected for increased wheel running also consumed more food when compared to control littersmates (Swallow et al. 1998, 2001a, b). The higher food intake results observed in the L-line (long term; experiment 1) compared to our previous experiment (Bunger et al. 2003) was most likely due to the increased physical activity caused by the introduction of wheels to the L line’s environment; the opportunity was given to display a novel behaviour. In contrast, the food intake of F mice did not change with altered activity patterns, as demonstrated by the regression analysis. This suggested F mice maintained their appetite even when running less. Longer studies would be needed to see if these long term changes would impact differentially on adiposity and weight gain as occurs when the mice are provided a high fat diet chronically (Morton et al. 2005). It is of interest that our studies show that only the L mice exhibit this correlation of physical activity and food intake. It is possible that with long term exercise L mice sense the threat to their lean mass (Blundell et al. 2003) as they have low fat stores to buffer against the negative energy balance caused by increasing exercise, and they compensate by increasing food intake. On the other hand reduced running activity does not change energy intake in the F mice, as has been found in humans (albeit over a shorter 7 day period) when placed on a more sedentary routine (Stubbs et al. 2004). However, since F and L mice both gained similar weight (20%), we cannot rule out changes in off-wheel activity energy expenditure (Sans-Fuentes et al. 2005; Buttner 1991; Hari et al. 1999). Similarly we cannot in the present study distinguish between relative gain in lean (e.g. muscle in high running L mice) versus fat (in increasingly obese F mice) mass.

In the older F and L mice, differences in home cage and running wheel physical activity as well as food intake were apparent immediately, consistent with previous studies showing that L mice ate more calories than F mice on both control and high fat diet (Morton et al. 2005). It is likely that the divergence in activity and food intake becomes more pronounced with age and is consistent with distinct genetically determined early and age-onset obesity mechanisms in this model (Stylianou et al. 2004).

Selection for higher (F line) or lower (L line) % body fat could also have resulted in a correlated response of decreased (F line) or increased (L line) wheel running activity. However, this seems unlikely in light of results obtained in mouse models selected for high or low wheel running activity (Swallow et al. 1998, 2001a, b). In most of these experiments, the wheel running selection test was applied at the age 42–56 days (similar to the period used in our running wheel experiment 1) but mice showed divergence very quickly and were selected based on a relatively short 6 day wheel running period (Koteja et al. 1999).

Since our mice started showing divergence in wheel running activity only after 19 days (3 days of acclimation period and 16 days of running wheel trial), we conclude that the wheel running behaviour trait was not a correlated response to the selection on high or low % body fat in our mice. While we cannot rule out that introduction of wheels may itself have stimulated physical activity differentially between the lines, our finding that home cage activity of L mice is significantly higher than in F mice without access to a wheel, indicates that increased (spontaneous) physical activity may indeed be an important genetic determinant of the divergent fat phenotype between the lines. That is, the L mice display higher activity in any given situation, and this makes them leaner.

It is of note that this and previous studies using defined low and high fat diets also demonstrated that L mice consume more calories than the F line (Morton et al. 2005). This makes the F line obesity distinct from that found in the polygenic New Zealand Obese (fat constitutes 40% of body weight) mouse (Jurgens et al. 2006) which exhibits pronounced hyperphagia as well as reduced core temperature relative to its lean comparator NZB strain (Jurgens et al. 2006). To test whether altered physical activity contributed to the divergent fat mass (Morton et al. 2005), we provided F and L mice with a running wheel and acclimated them (7 days) to a defined low fat diet (10.5% calories from fat) before exposing them for a further 7 days to a high fat diet (58% calories as fat). We noted that the lines exhibited novel and distinct strategies to regulate body weight when switched to the high fat diet. Lean mice reduced their caloric intake but continued to run at a higher rate than control fed F mice, with a trend towards weight loss. F mice on the other hand increased their running wheel
activity, and in fact matched the L line activity levels and maintained a similar caloric intake to the control diet phase. Despite this, some other metabolic response of increased energy efficiency occurs in the F mice, since they actually increased their body weight. Future studies will be needed to determine how this occurs, but it may involve reduced high fat diet-mediated energy dissipation within brown and white adipose tissue (Jurgens et al. 2006; Collins et al. 2001) and/or reduced energy expenditure off the running wheel (Swallow et al. 1998, 2001a, b). It is also of note that in the chronic running wheel experiment 1, F mice decreased their running whereas short term high fat feeding evoked a distinct increase in the activity level in the F mice, suggesting a specific response to the diet. Again this suggests that divergent adiposity in the F and L mice is driven by distinct genetic mechanisms from those found in the polygenic obese NZO model which has home cage spontaneous activity comparable to its lean controls (Jurgens et al. 2006). However, there are likely overlapping mechanisms since F mice and NZO respond similarly to running wheel stimuli by decreasing their activity when provided with this stimulus, at least on a normal diet (Jurgens et al. 2006). Future experiments that monitor behavioural traits directly in the home cage in combination with metabolic chambers to measure differences in fuel oxidation over the diurnal cycle will be needed to disentangle the ultimate behavioural distinctions between the F and L mice and provide a better basis for future quantitative evaluations of energy expenditure of physical activity and fuel utilisation.

The overall results of this study (two running wheel experiments and vertical rearing being significantly increased in L mice) are compatible with the earlier energy budget data which suggested the L line mice expend more energy on activity. Furthermore, we report novel alternative strategies between the F and L mice in response to high fat feeding that have direct relevance for the gene-environment interaction that contributes to human obesity in the face of caloric excess. One interpretation of this genetic predisposition to obesity is that it is an adaptive response reflecting a ‘thrifty genotype’ that was historically advantageous for surviving periods of famine (Neel 1998). However, this adaptive interpretation is at odds with the fact that modern humans include some individuals that have a strong genetic predisposition—whereas others show profound genetic resistance to—obesity (Loos and Bouchard 2003). This pattern is more consistent with a trait that is not under strong directional selection but is drifting under an absence of selective pressures (Speakman 2007). Individuals showing genetic resistance to obesity remain at normal weight in a wide range of obesogenic conditions. Our previous study (Morton et al. 2005) and the present study suggest that our F and L mice reflect these two extremes of susceptibility and resistance. It is likely that during the long-term genetic selection experiment, alleles have accumulated in the F lines which mirror the effect of gene mutations accumulated by drift in modern obese humans (though not necessarily the same genes). At the other extreme, obesity-resistant alleles have likely been preserved during the selection experiment in the L line. The metabolic advantage of reduced fat pad size in response to high fat feeding in L mice (Morton et al. 2005) remains an intriguing puzzle.

A frequently discussed strategy for future preventive health care, including obesity, proposes that patients will be sequence-tested for numerous genetic variants and on the basis of this counselled on possible disease prevention (Hunter 2005; Kaput et al. 2005). To achieve this, obesity-susceptibility or -resistant alleles need to be identified first. So far very few alleles of this type have been identified—one example is a stronger association between dietary fat intake and obesity in carriers of the Pro12Ala PPAR-γ gene allele (Memisoglu et al. 2003). Because such gene-environment interactions are very hard to study experimentally in humans owing to difficulties in controlling environment, our F and L mice represent a powerful resource for identification of such novel diet-responsive genes (possibly those underlying high fat induced voluntary physical activity in F mice and chronic high fat induced fat loss in L mice (Morton et al. 2005)). Whilst the mechanisms of gene selection that produced the F and L phenotype are clearly distinct from that of human populations, there may be potentially overlapping or related genetic mechanisms between the species that will provide insight into obesity-gene relationships in man. In relation to activity levels, recent studies show that humans who are prone to obesity have a propensity for maintaining postures that conserve energy (Levine et al. 1999, 2005). That is, the level of non-exercise activity (perhaps a correlate of our vertical posture allocation data) is a major determinant of weight gain, and that this is likely genetically determined in humans (Levine et al. 1999, 2005). The divergent responses to high fat feeding in F and L mice likely involve both exercise and non-exercise mediated mechanisms controlling calorie consumption (e.g. oxidation in muscle, fat or liver) and will potentially be a good model in which to identify novel genes underlying complex obesity and obesity resistance.

Acknowledgements We gratefully acknowledge support from the Slovenian Ministries (MVZT, MZ; young researcher programme), Agency ARRS (CRP V3-0365) and the ACERO Marie Curie Training site (Obeschool). We are grateful to the Scottish Executive Environment and Rural Affairs Department (SEERAD) for funding parts of this research. We thank John Verth, Moira Stewart and William Mungall from the mouse facilities at the University of Edinburgh for their help maintaining the selection lines and for animal husbandry. We also thank Gregor Gorjanc, Andrej Razpet, Paula Redman, Ela Krol and Tom Edie for their suggestions and help. Animals at the
University of Aberdeen were maintained by the animal house staff at the School of Biological Sciences to whom we are grateful. NMM is funded by a Wellcome Trust Career Development Fellowship. CJK is an MRC (UK) Senior Fellow. MH is funded by the Wellcome Trust, PS by a College Studentship (University of Edinburgh) and JRS by a Lloyds TSB senior research fellowship from the Royal Society of Edinburgh.

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