Coping styles and the pathophysiology of energy metabolism

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CHAPTER 6:

Forced and voluntary exercises counteracts hyperinsulineamia in rats: role of coping style.

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Abstract:

There are large individual differences in the succes rat of exercise intervention programs aimed at the prevention and treatment of obesity related disorders. In the present study we tested the hypothesis that differences in personality may have repercussion for the success rats of these intervention programs. To this end we tested the insulin signaling before and after voluntary wheel running, via intravenous glucose tolerance tests (IVGTT), in both passive (insulin resistant) Roman Low Avoidance (RLA) and proactive (insulin sensitive) Roman High Avoidance (RHA) rats. To control for the potential difference between voluntary and forced exercise, we also included RLA and RHA rats that were subjected to forced running. We found that 1) when given the opportunity to run voluntarily in a running wheel passive RLAs run more than proactive RHAs, 2) voluntary exercise lead to a normalization of the insulin response during an IVGTT in the RLA rats, and 3) there were no behavioral and physiological differences in efficacy between voluntary and forced running. We may thus conclude that exercise, either forced or voluntary, is a successful lifestyle intervention for the treatment of hyperinsulineamia in, especially, rats with a passive coping style.

1: Introduction:

There is hope that successful life style intervention can halt the ever increasing prevalence of metabolic disorders such as obesity, the metabolic syndrome and type 2 diabetes (1;2). Exercise-based intervention programs are particularly successful ((3;4), for a recent review see (5)). Exercise reduces body adiposity, improves glucose tolerance and increases insulin sensitivity (6-11). There are, however, large individual differences in the success rate of exercise intervention programs (12), which may be unrelated to the large individual differences by which subjects are susceptible to attract metabolic disorders in the first place (reviewed in (13)). There are nevertheless indications that both susceptibility to attract metabolic derangements as well as the efficacy of – and/or compliance to - exercise programs of the individual is associated to the personality type (14-16).

Differences in personality are a wide-spread phenomenon in the animal kingdom (17;18). This is, however, largely ignored in animal studies modeling the development of type 2 Diabetes, insulin resistance or the metabolic syndrome. In our most recent studies, we have filled in this gap using the Roman High and Low Avoidance rat selection lines (19-21). Rats from these selection lines differ in emotional reactivity and coping style (animal personality). Roman Low Avoidance (RLA) rats are highly emotional individuals with a passive coping style, Roman High Avoidance (RHA) rats are (pro)active rats with low emotional reactivity. They are also different at the level of several neuroendocrine, cardiovascular and metabolic parameters (20;22;23). In addition, the passive animals display, already at normal weight, several characteristics of the metabolic syndrome, such as hyperinsulineamia, visceral adiposity and hypertension (19-21). We have extended and confirmed these findings in outbred wild-type Groningen rats, in which personality type appeared to predict changes in metabolic profiles analogous to those observed in the RHA/RLA rats (19;21).

In the present study, we tested the hypothesis that differences in personality may have repercussions for the success rate of exercise interventions programs to normalize metabolic disorders such as type 2 Diabetes and the metabolic syndrome. Here we focus on the importance of differences in personality on the potential beneficial effect of exercise on hyperinsulineamia (and visceral adiposity) which clearly develop in the emotional reactive RLA rats. To this end, we performed a series of experiments in which the insulin response during an intravenous glucose tolerance test (IVGTT) was measured in both passive (insulin resistant) RLA and proactive (insulin sensitive) RHA rats. These IVGTTs were performed both under sedentary conditions and after 18 days of voluntary exercise in a running wheel. In most animal studies, exercise consist of voluntary running in a wheel. One should note,

however, that in humans, most exercise programs are not voluntary since they require motivated compliance of the individual. These compulsory exercise programs are, at least by a part of the participants, perceived as unpleasant, stressful and/or aversive. This means that there is a discrepancy between voluntary exercise in the rat model and forced exercise in the human. Therefore, to control for the potential difference between forced and voluntary exercise, we also included two groups of RHA and RLA that were subjected to 18 days of forced running.

2: Materials and methods

Animals:

The experiments were approved and checked by the local animal experimental welfare and care committee (DEC, Groningen, the Netherlands). Roman High (RHA) and Low (RLA) Avoidance rats, obtained from a breeding colony at the Clinical Psychopharmacology Unit (APSI) of the University of Geneva, were housed in a room controlled for temperature and humidity (20 ± 2 °C; 60%). The room was kept at a 12-12 hours light-dark cycle (lights on = CT0 at 01:00 hrs, lights off = CT12 at 13:00 hrs). The rats were fed a standard lab chow diet (*Hope Farms, RMH-B knaagdier korrel, Arie Block Diervoeding, Woerden, NL; 3.7 kcal/g, 14 % fat*). Food and water was available *ad libitum*.

Experimental design

The following experiments were performed: 1) Experiment 1 in which both RHA and RLA rats were submitted to an intravenous glucose tolerance test (IVGTT) at baseline and after 18 days of voluntary wheel running and 2) Experiment 2 in which both RHA and RLA rats were submitted to IVGTTs at baseline and after 18 days of forced wheel running. For both studies, the rats underwent surgery to place two indwelling jugular vein catheters for infusion and blood sampling (24). Rats were accustomed to the infusion and blood sample procedures before the onset of the experiments (25). The experiments started two weeks after surgery. Body weights and food take was measured daily throughout the experiment. The experimental design is summarized in figure 1.

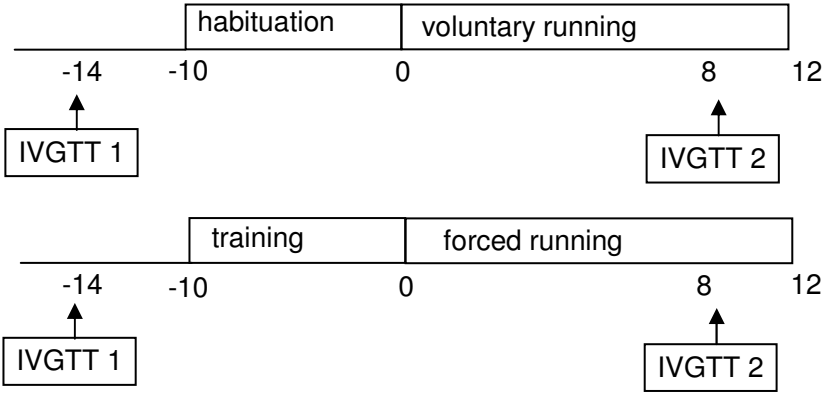

Figure 1: Experimental design. Top and bottom panels shows the experimental design for experiment 1, and experiment 2, respectively.

Experiment 1:

Twelve rats (6 RHA and 6 RLA) were housed in standard cages (24x24x32 cm). Two weeks after surgery, at day -14, a baseline IVGTT was performed. At day -10, the rats were transferred to standard running wheel cages (Nalgene polycarbonate running wheel cages [50-27-36 cm]) with free access to a running wheel (diameter 27cm, Mini Mitter, Oregon, USA). The rats were allowed to habituate to the wheel running for 10 days. During this habituation period running activity typically increases after which it stabilizes (Alfonso VM 2003). After habituation, the rats were allowed to run voluntary for 12 days (intervention period: day 0 until day 12). A second IVGTT was performed on day 8. Four days later, the rats were sacrificed for carcass analysis.

Experiment 2:

Sixteen rats (8 RLA and 8 RHA) were housed in standard cages (24x24x32 cm). A baseline IVGTT was performed at day -14. At day -10, the rats were transferred to forced activity cages (TSE, Bad Homburg). These cages contain running wheels with a diameter of 25 centimeter that force the animal to run. Both running speed and time spent on running are controlled. All animals were forced to run in a schedule that mimicked the voluntary running activity patterns of the pro-active RHA rats that participated in Experiment 1 (see Figure 3B). Since we observed that rats are running in bouts of circa 5 minutes, we decided to force the animals to run in a schedule of 5 minutes running and 5 minutes rest. The speed (max 20 m/min) was adjusted so that the total distance per hour was similar to that of the RHA rats. The rats were accustomed to the forced running paradigm for 10 days (day -10 until day 0). Intensity and duration was slowly build up, in parallel to the increasing running in the habituation period in the voluntary running animals in Experiment 1. During the intervention the rats were forced to run 5000 m/day. The forced activity intervention period lasted from day 0 until day 12. A second IVGTT was performed on day 8. Four days later, the rats were sacrificed for carcass analysis.

Intravenous glucose tolerance test:

At the day of an IVGTT, food was removed at the beginning of the light phase at CT 0. The IVGTTs were performed in the light phase, between CT 4 and CT 6. An IVGTT consisted of 30 minutes infusion of 15 mg glucose in 0.1 ml saline per minute (total 450 mg in 3 ml). Before the onset of the infusion, two baseline samples (0.2 ml) were taken at time points t = -11 and 1 minutes. The infusion of glucose was started at time point t = 0 minutes. Additional blood samples were taken at time points t = 5, 10, 15 , 20, 25, 30, 35, 40, and 50

minutes. A total blood volume of 2.2 ml was taken. Blood samples were kept on ice and stored in files with 10 µl EDTA (0.09g/ml). For glucose determination 50 µl of full blood with 450 µl Heparin solution (2%) was stored at -20°C until analysis. Blood glucose levels were determined using the ferry-cyanide method (26) in a *Technicon* auto analyzer. The remaining blood was centrifuged for 15 minutes and plasma was stored for insulin and corticosterone determination. Plasma levels of insulin and corticosterone were measured with commercial radioimmunoassay (RIA) kits (Linco Research and.M P Biomedicals).

Carcass analysis:

The rats were sacrificed four days after the last IVGTT. Three hours before lights off, rats were sacrificed using an overdose of pentobarbital. Epididymal and retroperitoneal fat pads and the liver were taken out and weighed. The skin with the subcutaneous fat was removed from the carcass. The liver, skin, and carcasses were dried at 80 °C for 5 days. The fat content was determined by extracting the fat from the tissue using a petroleum based Soxlet fat extractor. After fat extraction the tissue was dried for 5 days again. The relation between dry tissue weight before and after fat extraction provides information on the fat content of the tissue.

Data analysis:

Food intake and body weight data are presented as daily averages with standard error of mean (sem). Average running wheel activity was calculated as averages from day 0 until day 12 for each individual animal. Glucose and insulin levels are presented in group averages with standard error of mean. Statistical differences between groups were determined with repeated measures ANOVA followed by Tukey post-hoc test using coping style and type of intervention as between subjects factors and time of measurement as within subjects factor. The area under the curves (AUC) of the insulin responses were calculated and averaged. Percentage fat mass was calculated by dividing total dry fat mass by total dry lean body mass and multiplying this with 100%. Fat mass and weight of the different fat pads are presented as group averages with standard error of the mean. Differences in the area under the insulin response curve, and body composition were statistically tested with one-way ANOVA followed by Tukey post hoc analysis using coping style and type of intervention as the between subject factors. All statistical analysis used a 5% confidence interval.

3: Results:

Figure 2 displays body weight gain and food intake of the different groups during the intervention period from day 0-12. There were no differences in neither food intake nor body weight gain between any of the groups. In all groups, food intake was on average higher during the intervention period when compared to the intake during the baseline period (baseline: 101 ± 4.8 kcal/day; intervention: 120 ± 5.6 kcal/day; $F(1, 28) = 4.562$ $p<0.05$). Figure 3A displays the running activity of all groups. In Experiment 1, RLA rats ran significantly more than RHA rats ($F(1,15) = 9.332$) $p< 0.01$) .

Blood glucose and plasma insulin levels are presented in Figure 4. There were no significant differences in blood glucose levels between the groups. Insulin responses were significantly different ($F(5,39) = 6.294$, $p<0.01$): 1) at baseline, since RLAs have much higher levels than RHAs ($p<0.01$). 2) in the RLAs, when the higher baseline levels are compared to the much lower levels of insulin after 18 days of both voluntary and forced exercise (voluntary running $p<0.01$; forced running $p<0.01$) 3) in the RHAs when baseline levels are compared with the somewhat lower insulin levels after 18 days of voluntary but not forced exercise (voluntary running $p<0.05$; forced running $p = 0,103$). There were no differences in plasma insulin levels when the responses in the voluntary runners were compared with those of the forced runners, both under baseline conditions and after 18 days of exercise

Peak circadian corticosterone levels were not different between the forced and voluntary running rats under any circumstances (RLA baseline: 250 ± 35.3 ng/ml; RHA baseline: 225 ± 29.3 ng/ml; RLA voluntary running: 242 ± 29.7 ng/ml; RHA voluntary running: 226 ± 25.3 ng/ml ; RLA forced running: 263 ± 29.7 ng/ml; RHA forced running : 233 ± 25.26 ng/ml). Baseline levels of corticosterone at circadian peak level tended to be higher in the passive RLAs when compared to the proactive RHAs but this difference did not reach statistical relevance.

Carcass analysis showed there were no differences in the percentage of body fat at the end of the study (RLA voluntary running: 35.9 ± 0.47 %; RHA voluntary running: 34.9 ± 0.54 %; RLA forced running: 33.3 ± 0.56 %; RHA forced running: 33.1 ± 0. 62 %). The distribution of body fat was however different between the groups: passive RLAs have relatively more fat in the epididymal depot in comparison to proactive RHAs (RLA voluntary running: 4.42± 0.24 g; RHA voluntary running: 3.91 ± 0.21 g; RLA forced running: 5.8 ± 0.49 g; RHA forced running: 3.6 ± 0.26 g; ($F (3,25) = 6.426$ $p<0.05$)). There were no differences between RLAs and RHAs in the amount of fat distributed in the retroperitoneal fat depot (RLA voluntary running: 7.7 ± 0.77 g; RHA voluntary running: 7.1 ± 0.69 g; RLA forced running: 8.1 ± 0.76 g; RHA forced running: 7.6 ± 0.84g).

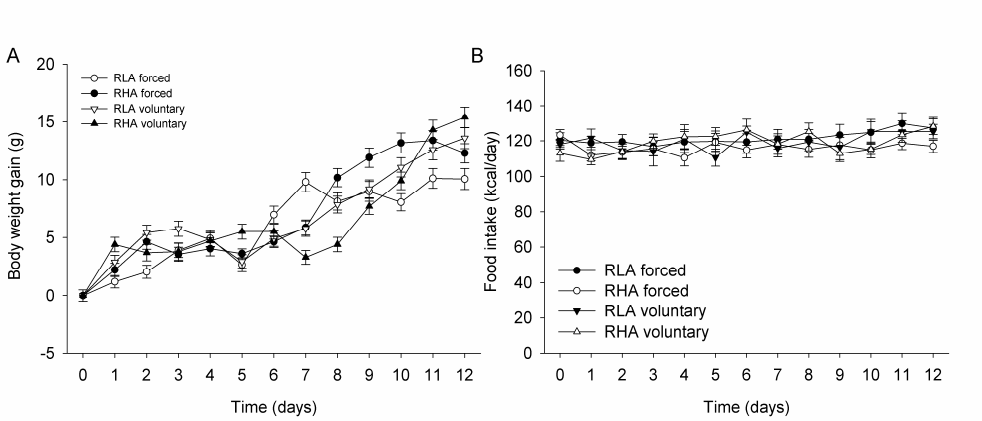

Figure 2: Body weight gain and food intake of passive and proactive rats that ran voluntarily or forced. Black circles = proactive forced runners (n=8), white circles = passive forced runners (n=8), black triangles = proactive voluntary runners (n=6), white triangles = passive forced runners (n=6).

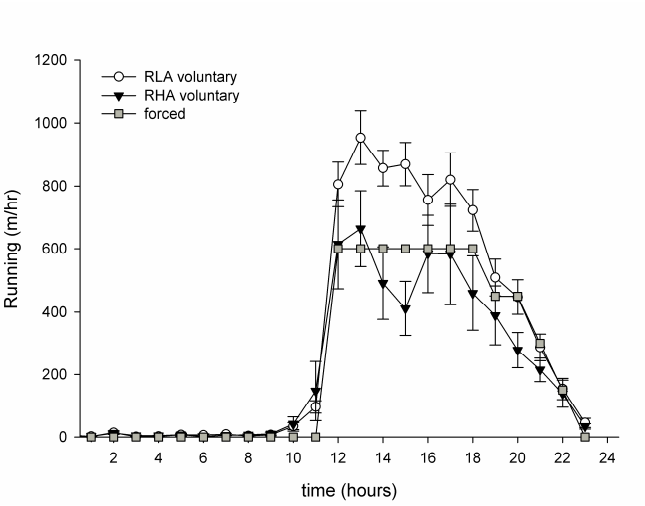

Figure 3: Running activity in experiments 1 and 2 rats. White symbols = voluntary running RLA rats, Black symbols = voluntary running RHA rats, Grey symbols = forced running rats of both strains * indicates a significant difference between voluntary running RLAs and RHAs.

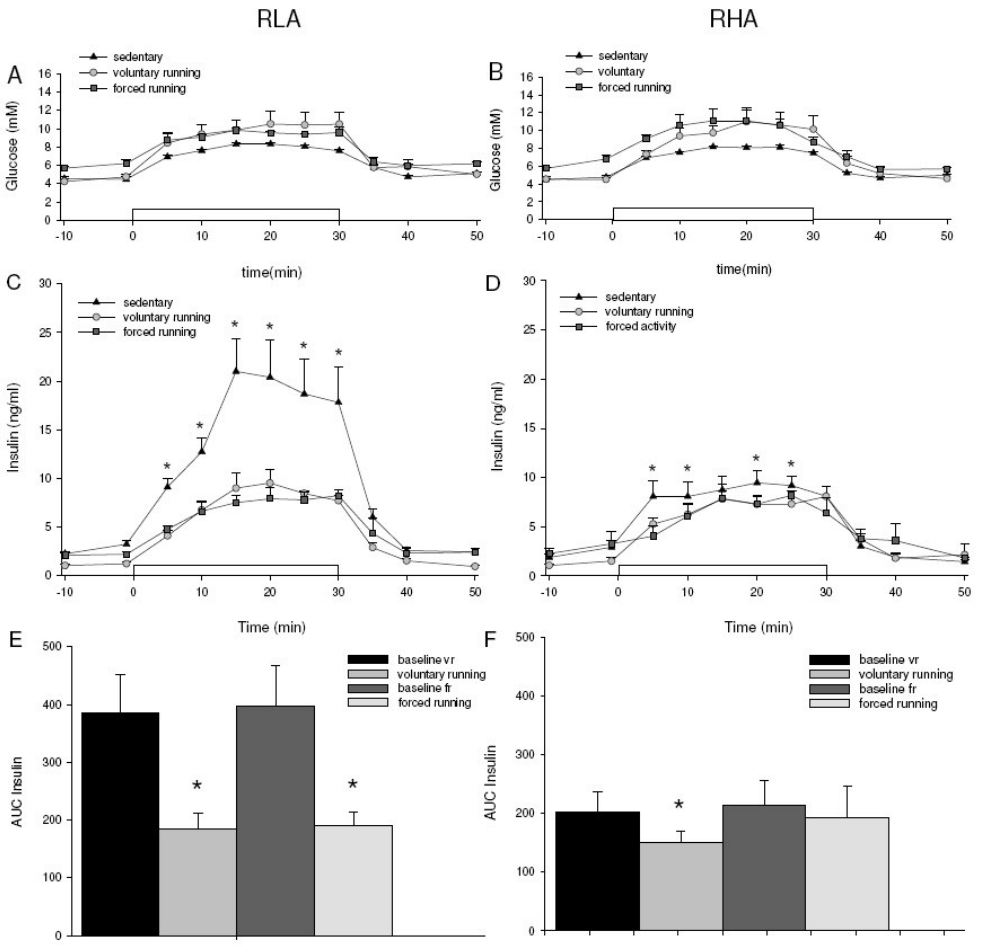

Figure 4: Glucose (A and B) and insulin (C and D) levels before, during and after an IVGTT at baseline and after 18 days voluntary or forced running in both RLAs (A and C) and RHAs (B and D). Baseline values in experiment 1 and 2 are combined in one graph. Black triangles = baseline, light grey circles = voluntary runners, dark grey squares= forced runners. Area under the insulin curve at baseline and after 18 days of running in voluntary and forced running RLA (E) and RHA (F) rats. Black bars = baseline voluntary runners, medium grey bars = 18 days voluntary running, dark grey bars = baseline forced runners, light grey bars = 18 days forced running. vr = voluntary running, fr = forced running

* indicates a significant difference between baseline conditions and both voluntary running and forced running conditions.

4. Discussion

The aim of the current study was to investigate the interaction between personality and exercise in relation to the treatment hyperinsulineamia. The major findings of this study were that 1) when given the opportunity to run voluntarily in a running wheel passive RLAs run more than proactive RHAs, 2) voluntary exercise lead to a normalization of the insulin response during an IVGTT in the insulin resistant RLA rats, and 3) there are no behavioral and physiological differences between voluntary and forced running. Consistent with our previous studies, passive RLA rats displayed a much higher insulin response to an intravenous glucose tolerance test than found in the RHA rats (20). Exercise completely normalized this elevated insulin to control levels indicating that exercise is indeed a successful life style intervention for the treatment of hyperinsulineamia, in particular in insulin-resistant rats with a passive coping style.

The most interesting finding is the increased running activity in the passive RLA rats when they were allowed to run voluntary. This is remarkable since the so-called passive rats were, in other behavioral conditions, characterized as having lower locomotor activity, for example in behavioral tests such as the open field test, the Porsolt forced swim procedure and the elevated plus maze test (27-29). However, these tests are all short term responses to unfamiliar conditions, whereas in our study we monitor the internal motivation to be active in a familiar environment.

The increased running in the RLAs resulted in the normalization of the insulin response during an IVGTT, which is a strong indication of improvement of insulin sensitivity. Such a phenomenon, i.e. increased spontaneous wheel running activity in metabolically deranged rodents has been reported before, among others in overweight animal models such as the Oleft rat (30) and the MC4 knockout mouse (31). Both the Oleft and the MC4 knockouts have an obese and insulin-resistant phenotype under sedentary conditions, but compensate for this by increased activity when allowed to run spontaneously in wheels, leading to normalization of their body weight. In the present study, we observe that presumably insulin resistant rats show increased running to normalize their insulin sensitivity. Therefore it is tempting to speculate that the increased running may be considered as a behavioral strategy to compensate for the reduced insulin sensitivity in the sedentary state. Like insulin, muscular contractile activity causes glut-4 translocation and increases glucose uptake (32), hence exercise would benefit RLA rats more than RHA rats. Along these lines, it may be speculated that exercise has a larger impact on glucose availability to neuronal circuitry (33) in RLA rats than in RHA rats, which might be a mechanisms by which to RLA rats sustain a higher level of running wheel activity than RHA

rats. Another implication of these results is that the sedentary state, at least in rodents, should not be considered as a the proper control condition because physical activity and health are inevitably linked (34). A point that is illustrated by the healthy insulin profiles in the voluntary running RLA rats.

We argued in the introduction section that the translational value of a voluntary exercising animals might be limited, because humans subjected to exercise-based interventions can perceive it as a stressful workload. Our second study therefore investigated difference in the efficacy of forced and voluntary exercises in the RLA rats. We showed that both forced and voluntary running resulted in “normalized” insulin responses to an IVGTT in the RLA rats. This suggests that the exercise itself rather than the voluntary or forced nature of the running determines the beneficial effects of the wheel running on insulin sensitivity. In the current study, the amount of forced running was based on the average voluntary running activity of proactive RHA rats. Proactive rats were shown to run less voluntarily than passive rats. Since this forced running improved insulin signaling in the RLA rats one may argue that the amount of running might not be crucial for the attenuation of hyperinsulineamia in the RLAs. One should, however, be somewhat cautious in comparing the effects of voluntary and forced activity, since a higher demand on contractile activity and gate coordination can not be ruled out in animals subjected to forced running.

The current set-up was chosen to minimize the stress of the forced running paradigm, especially since it might be perceived differently in RLA and RHA rats. A difference in perception of the workload imposed on them might, however, prove important when studying exercise based lifestyle interventions. In humans it is argued that individuals with proactive personality traits have a lower perception of exertion and endure higher amounts of exercise than individuals with passive personality traits (*Hassmen, Stahl &* Borg 1993). Nevertheless, the observation that there are no behavioral and physiological differences between voluntary and forced running animals, strengthens the face validity of the voluntary rat model for translation to human studies.

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