Individual variation in the (patho)physiology of energy balance

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ABSTRACT

There are large individual differences in the susceptibility for metabolic disorders such as obesity, the metabolic syndrome and type 2 diabetes. Unfortunately, most animal studies in this field ignore the importance of individual variation which limits the face validity of these studies for translation to the human situation. We have performed a series of studies that were particularly focused on the individual differences in the (patho)physiology of energy balance. The studies were performed with passive and proactive individuals of two different rat strains: the Roman High and Low Avoidance rats and the Wild type Groningen rat. The data reveal that passive and proactive individuals differ significantly on several parameters, i.e. body composition, Hypothalamic–Pituitary–Adrenal (HPA) axis activity, plasma levels of insulin and leptin, intestinal transit time, systolic blood pressure and meal patterns. We also found that the selection line of the Roman Low Avoidance rat may be considered as a non-obese animal model for the metabolic syndrome, since these rats display, under sedentary conditions, many of the related symptoms such as hypertension, visceral adiposity and insulin resistance during an intravenous glucose tolerance test. These symptoms disappeared when the animals were allowed to exercise voluntarily in a running wheel. We conclude that experiments with passive and proactive individuals are highly relevant for studying the (patho)physiology and behavior of energy balance and the related metabolic disorders.

1. Personality in energy balance

In humans, there are large individual differences in the susceptibility for metabolic disorders such as obesity, the metabolic syndrome and type 2 diabetes. Unfortunately, most animal studies in the field ignore the importance of individual variation and use standard models such as the Wistar and the Sprague–Dawley rats. This limits the face validity of rat studies in energy balance and metabolism for translation to the human situation.

Variation in the susceptibility of metabolic disorders between individuals is, in part, caused by psychosocial factors such as differences in levels of education, low sense of coherence, work stress and lack of sleep [1–3]. Personality plays a role as well, as indicated by observations in children [4] and adults [5] that a type A personality has a higher risk to develop the metabolic syndrome. The following paragraphs are therefore particularly focused on our studies on the role of an individual's personality in the (patho)physiology of energy balance.

2. Personality and coping style

Personality is defined as a set of behavioral and physiological responses that characterize an individual. Most of the current theories on personality are based on the behavioral disposition model proposed by Allport in 1937 [6]. This theory states that a personality characterizes individuals in terms of a set of stable dispositions (traits) that are distinctive for the individual and determine a wide range of behavioral responses. A personality is thus a set of behavioral responses that are genetically imbedded within an individual and these behavioral strategies are employed throughout life to interact with the environment. Even though the theoretical framework of the behavioral disposition model is well accepted, scientific descriptions of personality are mostly methodology based; the methods used to assess personality (Bortner scale, Big Five, and Jenkins Activity Survey) also determine the definition of personality [7–9].

This makes it difficult to investigate the physiological and neuronal mechanisms underlying the behavioral differences between individuals. The use of an objective and unbiased animal model might be useful for this since most described personality traits in humans are identifiable in animal models as well [10–12]. However, the animal literature prefers the term coping style when describing individual differences in physiology and behavior. Coping style is defined as a coherent set of behavioral and physiological stress responses that are

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consistent over time and that characterize a certain group of individuals [13]. Although the definitions of personality and coping style show overlap, coping style may be preferred for translational studies since it is based on an objective observation of behavior and physiology.

3. Proactive and passive coping styles

The development of a coping style is aimed at controlling the environment successfully to increase survival [14]. In most animal species, including humans, two major coping strategies (proactive and passive copings) are distinguished (*reviewed in Ref.* [13]). The proactive coping style is characterized by the fight/flight response, a stress response primarily mediated through activation of the sympathetic nervous system (reflected by increased catecholamine release [15]). As a consequence, heart rate, blood pressure and blood flow to the muscles are increased allowing the animal to escape or fight the threat [16]. Individuals with a proactive coping style display higher levels of aggression, impulsivity and they are more prone to routine formation [17,18]. Proactive individuals are also characterized by low hypothalamus–pituitary–adrenal (HPA) axis reactivity [19–21].

The passive (or reactive) coping style originates from a conservation/withdrawal response. This response, originally described by Engel et.al [22], is characterized by freezing behavior, activation of the HPA-axis (leading to elevated corticosterone/cortisol levels), increased parasympathetic and reduced sympathetic reactivity [19–21]. The freezing response minimizes the chance of being attacked and thereby lowers the risk of being harmed by the threat. Individuals with a passive coping style are characterized by a low aggressive nature, low levels of cue dependency and high levels of behavioral flexibility [17,18,23].

In wild populations of animals, the coping strategies of the individuals within a population display a bimodal distribution, mainly because individuals with an extreme coping style have by definition a higher fitness [14,24,25]. The evidence for this is derived from studies in wild populations of the great tit (*parus major*) and the mouse (*mus musculus domesticus*) [17,26]. Individuals with an intermediate coping style are generally not present in a population in the wild since they have a lower fitness in both a stable, territorial environment as well as in a variable migratory setting. In contrast, in a laboratory or domestic settings there is no environmental pressure pushing the population into a bimodal distribution. This means that in domesticated populations, like domesticated pigs and most laboratory rat strains, a normal distribution in coping strategies is observed [24].

4. Proactive and passive rat models

Studies in our laboratory focus on the individual differences in the (patho)physiology and behavior of energy balance and its impact on the development of metabolic disorders, such as obesity and insulin resistance. In these studies we use two rat models with extreme coping styles: 1) Roman High and Low Avoidance rats and 2) proactive and passive Wild Type Groningen rats.

The Roman High and Low Avoidance rat selection lines were founded in 1965 by Bignami [27] and populations of these rat lines have been maintained ever since. The rats originated from a Wistar stock and have been selectively bred on the basis of their performance in a two way active avoidance test. During this test the rats are placed in a shuttle box with two compartments and trained to associate a light stimulus with a mild foot shock. This shock can be avoided by moving to the other compartment of the box [27]. The initial acquisition of avoidance behavior is strongly dependent on emotional reactivity, anxiety and coping strategy [28,29]. The Roman High Avoidance (RHA) rats were selectively bred on the basis of rapid learning to avoid the shock, while the Roman Low Avoidance (RLA)
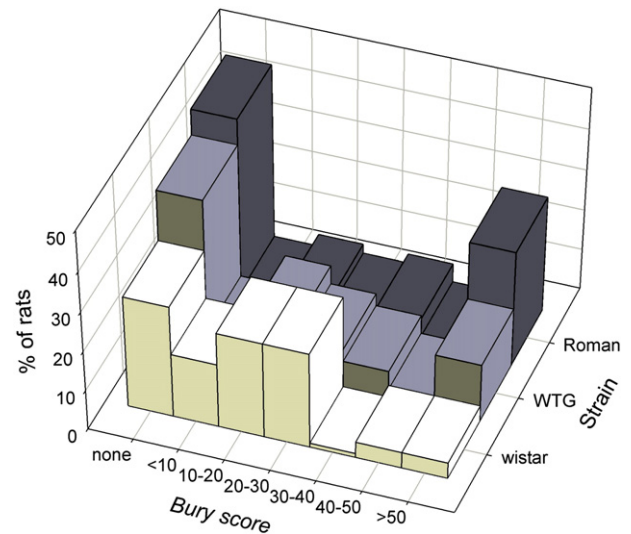
Fig. 1. Distribution of bury behavior within the Roman, Wild Type Groningen (WTG) and Wistar rat populations. The z-axis presents the three rat populations tested. On the x-axis the percentage time spent burying during a defensive bury test is categorized. On the y-axis the percentage rats of each population in each category is presented. The defensive bury test was performed using methods described in Ref. [36].

rats were selectively bred based on non-acquisition of avoidance behavior. RLA and RHA rats are extensively studied in the field of aggression, anxiety and depression and several excellent reviews are available that provide information on the behavioral, hormonal and neuro-chemical characteristics of these passive and proactive selection lines [30–32].

The Wild Type Groningen (WTG) rat population is originally derived from the University of Wageningen in The Netherlands and is bred in our Department under conventional conditions. This outbred rat population is characterized by display of a wide variety of behavior and the bimodal distribution of passive and proactive individuals, as observed in the wild populations, is still present in the WTG rat (for a review, see Ref. [33]).
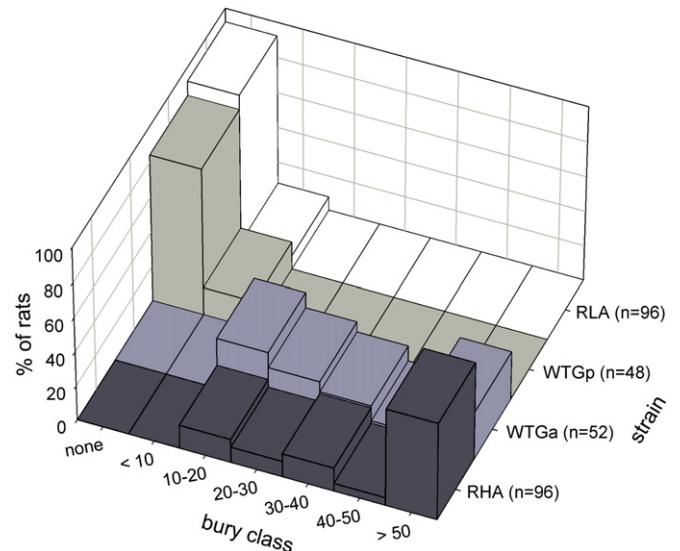
Fig. 2. Distribution of bury behavior in Roman Low Avoidance (RLA), Roman High Avoidance (RHA), passive Wilde Type Groningen (WTGp) and proactive Wild Type Groningen (WTGa) rats. The z-axis presents the four rat strains tested. On the x-axis the percentage time spent burying during a defensive bury test is categorized. On the y-axis the percentage rats of each population in each category is presented. The defensive bury test was performed using methods described in Ref. [36].

Table 1

Behavioral characteristics of passive and proactive rats from the Roman and the Wild Type Groningen strains. The defensive bury test was performed using methods described in Ref. [36]. The Elevated plus maze test was performed according to the methods described in Ref. [82]. The open field test was performed using methods described in Ref. [83]. The Novel object recognition task was performed using methods described in Ref. [84]. Differences between the proactive and passive rats within each strain were assessed with a one-way ANOVA (coping style = between subjects factor).

	RLA	RHA	WTGp	WTGa
Proactive behavior				
Defensive bury test (% immobile)	65.0 ± 9.9	1.5 ± 1.0[*]	75.0 ± 9.1	13.2 ± 4.7[*]
Defensive bury test (% bury prod)	1.5 ± 0.9	63.7 ± 7.8[*]	0.8 ± 0.8	38.7 ± 7.9[*]
Defensive bury test (% explore cage)	8.0 ± 3.2	16.1 ± 6.2	15.7 ± 6.1	25.6 ± 3.8
Defensive bury test (% explore prod)	12.7 ± 10.2	13.3 ± 4.1	6.4 ± 2.6	10.2 ± 3.3
Defensive bury test (% grooming)	12.4 ± 4.0	9.0 ± 4.0	6.6 ± 2.7	12.2 ± 4.9
Anxiety				
Elevated plus maze test (% open arms)	6.2 ± 3.5	12.1 ± 2.1[*]	10.5 ± 2.3	11.2 ± 1.5
Open field test (% inner zone)	10.2 ± 4.5	12.4 ± 1.3	11.7 ± 1.9	14.2 ± 1.5
Learning and memory				
Novel object recognition (% new object)	69.5 ± 3.2	68.9 ± 2.6	No data	No data
Aggression				
Resident intruder test (attack latency in second)	432.2 ± 75.2	124 ± 43.87[*]	497.2 ± 55.2	76.6 ± 38.0[*]

[*] Indicates significant differences between the passive and proactive rats within the same strain (p<0.05).

In our laboratory we use the so-called defensive bury test, developed by Pinel and Treit [34], to select proactive and passive individuals within the WTG rat population. Rats that display less than 10% (in time) burying behavior are categorized as passive, individuals that spend more than 20% of the time burying are categorized as proactive. Fig. 1 shows the distribution of proactive and passive coping behavior in the defensive bury test for individuals from the Roman selection lines, the WTG rats and a standard Wistar population. It is evident that the bimodal distribution in coping style is still present in both WTG rats and the Roman lines. This is not the case in the standard Wistar rat line in which most of the animals can be characterized as passive or intermediate individuals, an observation that confirms previous findings in the so-called resident–intruder aggression test [35].

Fig. 2 shows the distribution in bury behavior of the Roman selection lines and the WTG rats that were used in our studies. It is clear that, in comparison with the WTG rats, the RHA rats are more extreme in their proactive behavior, which confirms our previous findings. Likewise, passive RHA rats tended to bury less than passive WTG rats. This difference did, however, not reach statistical significance [36].

Table 1 provides the results of a series of additional behavioral tests in both rat strains to confirm the passive and proactive nature of the rats that were used in our experiments. In both rat strains, the passive personalities may be characterized as non-active and non-aggressive with relative high levels of anxiety while proactive animals are much more active and aggressive with low levels of anxiety. The data confirm previous studies from others [13,28,29,35].

5. Coping style and energy balance

So what is the influence of the coping style of an individual on the (patho)physiology of energy balance? Tables 2 and 3 summarize some of the data obtained. Under baseline conditions (ad lib standard lab chow and resting state) there were no differences between passive and proactive animals with respect to body weight, 24 h food intake, energy expenditure and blood glucose levels. There were, however, significant differences in body composition: passive rats had more fat in the visceral fat depot in comparison with proactive rats [37]. These changes in body composition were accompanied with related changes in the regulatory hormones: both plasma insulin, leptin and corticosterone levels were higher and sympathetic outflow (reflected by plasma noradrenalin levels) was lower in the passive individuals [36]. The differences between passive and active animals were all highly significant in the Romans selections lines and less pronounced in the WTGs. The observed effects on plasma noradrenalin and corticosterone confirm the idea that the proactive coping style is characterized by increased activation of the sympathetic nervous system in combination with low hypothalamus–pituitary–adrenal (HPA) axis (re)activity and that passive individuals are characterized by increased activation of the HPA-axis and reduced sympathetic (re) activity [13,19–21].

One of the most striking findings was the difference in baseline plasma insulin levels between RHAs and RLAs. Passive individuals have high insulin levels and proactive animals have relative low levels of plasma insulin under baseline conditions. Fig. 3 shows the

Table 2

Metabolic characterization of a coping style. An arrow indicates a significant difference from controls, "ns" no differences to controls [49].

	RLA	RHA	WTGp	WTGa
Body weight (g)	ns	ns	ns	ns
Food intake (kcal)	ns	ns	ns	ns
Resting energy expenditure	ns	ns	ns	ns
Fat mass (%)	ns	ns	ns	ns
Fat free mass (%)	ns	ns	ns	ns
Epididymal fat (g)	↑	↓	↑	↓
Retroperitoneal fat (g)	↓	↑	↓	↑
Subcutaneous fat (g)	ns	ns	ns	ns

Table 3

Levels of regulatory hormones in passive and proactive Roman rats. Hormone levels were measured in blood samples taken from indwelling jugular vein catheters. The samples were taken in the middle of the light phase (CT4–CT6) from animals that were fasted for at least 4 h. Differences between the RLA and RHA rats were assessed with a one-way ANOVA.

	RLA	RHA
Blood glucose (mmol)	6.07 ± 0.30	6.56 ± 0.32
Plasma insulin (ng/ml)	2.76 ± 0.56[*]	2.06 ± 0.25
Plasma leptin (ng/ml)	4.46 ± 0.65[*]	3.19 ± 0.76
Plasma corticosterone (ng/ml)	364.4 ± 42.1[*]	228.5 ± 30.9
Plasma noradrenalin (pg/ml)	78.1 ± 25.6[*]	178.7 ± 11.9

[*] Indicates a significant difference between RLA and RHA rats (p<0.05) (derived from Refs. [17] to [49]).
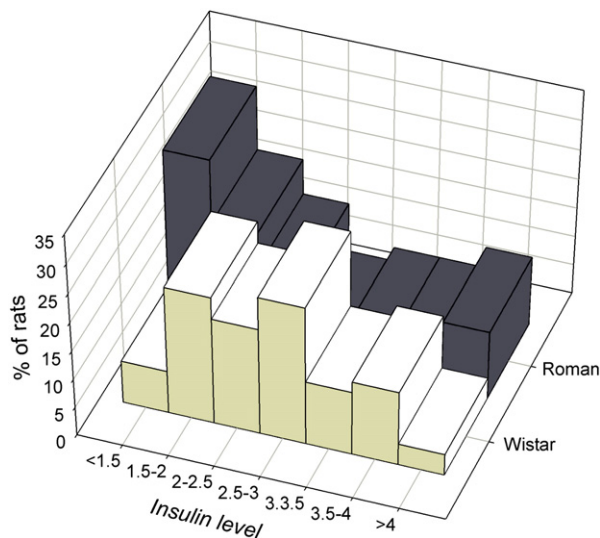
Fig. 3. Distribution of insulin levels within a Roman and a Wistar rat population. The z-axis presents the two rat populations tested. The x-axis expresses baseline insulin levels at the middle of the light phase, the y-axis the percentage rats of each population in each category. Insulin was measured in blood samples taken from indwelling jugular vein catheters. The samples were taken in the middle of the light phase (CT4–CT6) from animals that were fasted for at least 4 h.

Fig. 4. Blood glucose and plasma insulin levels during to a 30 min intravenous glucose infusion (15 mg/ml) in Roman Low Avoidance (RLA, n = 8), Roman High Avoidance (RHA, n = 8), passive Wilde Type Groningen (WTGp, n = 8) and proactive Wild Type Groningen (WTGa, n = 8) rats. All rats were fed a standard lab chow diet and housed under sedentary conditions. Differences between the four experimental groups were assessed by calculating the area under the response curve followed by ANOVA statistical analysis (experimental group = between subjects factor). * indicates a significant difference between RLA and RHA, WTGa, WTGp rats (ANOVA F [3,28] = 9.368 p<0.01).
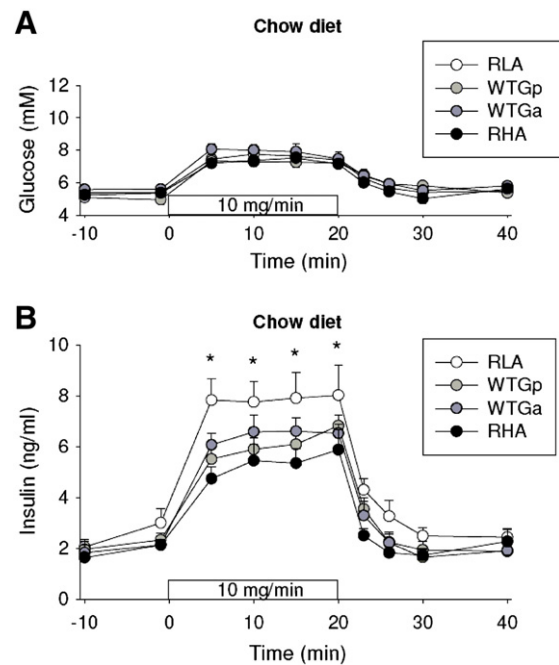Data from Boersma 2010 [36].

distribution of plasma insulin levels over the Wistar and Roman rat population. There is a clear bimodal distribution within the Roman rat population which is remarkably different from the normal distribution in the standard laboratory Wistar rat strain. This suggests that plasma insulin levels are strongly linked to the coping strategy of an individual.

We further investigated this by subjecting passive and proactive individuals from both strains to a series of intravenous glucose tolerance tests (IVGTT). Fig. 4 shows that passive RLA animals are not only characterized by increased baseline insulin levels but also by an elevated insulin response during an IVGTT, suggesting that these animals are insulin resistant, even on standard chow diet [37]. This development of insulin resistance was, as mentioned before, accompanied with an elevation in baseline plasma leptin levels and increased epididymal fat deposition (visceral obesity). The passive WTG rats had also an increased insulin response to an IVGTT, but unfortunately this did not reach significance. That is, when the animals were fed a standard chow diet. But when we placed them on a palatable high fat diet, the passive individuals clearly developed insulin resistance (reflected by an elevated insulin response to an IVGTT) and visceral obesity [36]. A passive personality may therefore be considered as a risk factor for developing insulin resistance. In contrast, proactive individuals of both strains were always resistant to the development of insulin resistance and visceral obesity, even when they were overfeeding on a palatable high fat diet.

6. Coping style and cardiovascular activity

In a subset of experiments, passive and proactive animals from the Roman selection lines were equipped with the Dataset telemetry devices [15] for chronic on-line measurements of blood pressure and heart rate. The data under resting conditions are presented in Table 4.

Systolic blood pressure is dramatically increased in RLA rats and the increased pulse pressure indicates that this, in part, may be caused by increased arterial stiffness. From these data, one may conclude that the passive RLA rat, which is already characterized by insulin resistance and visceral obesity, is also hypertensive. This means that the RLA rat develops several characteristics of the metabolic syndrome, already under baseline chow conditions. Since there are

no differences in food intake and body weight between RLAs and RHAs (Table 2), one may argue that the RLA rat may serve as an ideal animal model for the metabolic syndrome in non-obese animals.

The underlying mechanism that may explain the increased blood pressure in the passive animals is still unknown. In a previous study [37] we found that Table 2 also reveals that RLAs are characterized by elevated daily water intake. The combination of increased blood pressure and elevated water intake points to a possible role for

Table 4

Cardiovascular parameters in passive and proactive Roman rats. Before the start of the experiment, the rats in this study were equipped with a transmitter (PA-C40, Data science, St. Paul, MN) to allow cardiovascular measurements. During the whole study the cages of the rats were place on an antenna board (RA1010, Data sciences) to allow constant measurements of cardiovascular parameters. The data were collected and analyses using Dataquest IV (Data sciences) software. Cardiovascular parameters were measured once every 5 min for 10 s. The cardiovascular parameters of the rats were measured for one week (days 0–7) under the standard sedentary conditions in rats fed either a chow or a high fat diet. For statistical analysis an average per week was calculated for each rat. Differences between RLA and RHA rats were assessed with a multivariate ANOVA (strain = between subjects factor).

	RLA	RHA
Chow		
Heart rate (bpm)	285 ± 5.1	291 ± 6.4
Systolic pressure (Hg/mm)	120 ± 3.5	109 ± 1.3*
Diastolic pressure (Hg/mm)	83 ± 2.9	78 ± 2.4
Pulse pressure (Hg/mm)	36.9 ± 1.7	31.0 ± 2.7*
Heart rate variability	4.9 ± 0.3	5.1 ± 0.5
High fat diet		
Heart rate (bpm)	289 ± 5.1	290 ± 6.5
Systolic pressure (Hg/mm)	126 ± 2.4	113 ± 3.6*
Diastolic pressure (Hg/mm)	86 ± 1.7	86 ± 5.0
Pulse pressure (Hg/mm)	39.9 ± 1.6	29.2 ± 6.5*
Heart rate variability	5.2 ± 0.2	5.1 ± 0.3

* Indicates a significant difference between RLA and RHA rats (p<0.05) [50].

vasopressin are discussed in Refs. [38,39]. Indeed, Aubry and colleagues showed already in 1995 that vasopressin levels at the level of the paraventricular nucleus of the hypothalamus are increased under basal conditions in the passive RLAs [40].

7. Coping style and eating behavior

Data from a literature suggest that there are marked differences in the eating behavior of passive and proactive personalities, in humans as well as in experimental animals [41,42]. Rossi et al. studied different aspects of eating behavior of the Roman rat strains and found that the passive and proactive individuals are different with respect to meal size, eating rate (the number of kcal/min consumed within a defined meal) and distribution of meals over the day [41]. They also found that RLAs eat more frequent meals with a smaller meal size and a low eating rate, with relatively more meals in the inactive light period. In our studies we confirmed Rossi's findings in the Romans and, in part, in the WTGs (Table 5).

One may argue that the nature of the personalities by itself may explain the differences in eating behavior. Passive animals are more anxious and more sensitive to environmental cues [29] and are therefore more easily distracted when eating. Distraction and anxiety leads to a lower eating rate and smaller meals and, consequently, to an increased number of meals per day. The observation that RLAs but not RHAs eat less in a novel environment strengthens this idea. Likewise, the higher eating rate and, to a lesser extent, the larger meals in the RHA and active WTGs fits with the rigid nature of proactive animals.

Physiological differences, in particular at the level of the gut hormones, are known to influence meal patterns and many aspects of eating behavior. Unfortunately, data on the individual variation of the actions and alterations of gut hormones such as CCK and Ghrelin are not available in the literature. We measured the intestinal transit time in RLA and RHA rats and obtained indirect evidence that gut factors might be different between proactive and passive individuals. In short, a sucrose solution with a dye (carmine red − E120) was given to the animals, they were sacrificed 30 min later and the concentration of the dye was measured by photo-spectrometry in the different segments of the gut [43]. Fig. 5 reveals that intestinal transit time was significantly shorter in the passive RLAs when compared with the proactive RHAs. Although this is contradictory to what should be expected on the basis of lower eating rate and smaller meal size in passive animals, the striking differences in meal patterns and intestinal transit time between passive and proactive individuals make it worthwhile to perform future studies on individual differences in gut physiology.

In their study in 1997, Rossi et al. [41] observed that there were differences between RLAs and RHAs with respect to the distribution of meals over the day. This seems to be caused by differences in the biological clock since Rivest and colleagues found that the pineal of the RHAs was twice the size of the glands of the RLA rats [44]. This difference at the level of the pineal gland leads to increased secretion of melatonin in the RHA rats [45] and significant changes in sleep patterns and the organization of sleep [46]. We also looked at the
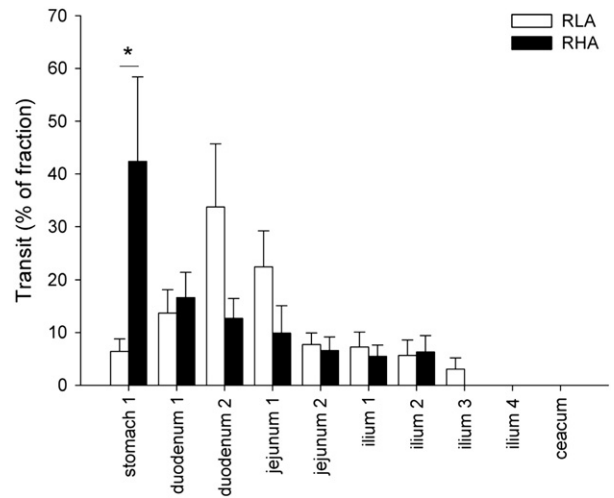
Fig. 5. Intestinal transit time of RLA and RHA rats. Transit of a red dye (carmine red) 25 mg/100 ml diluted in 10% glucose solution was measured 30 min after ingestion. The small intestine (including stomach and ceacum) was dissected directly after sacrifice. First, the stomach was isolated by placing ligatures at the end of the esophagus and the pylorus. Here after the duodenum was isolated and divided into two equal parts. Next the jejunum was isolated and divided with ligatures into two parts. Hereafter the Ilium was isolated and divided into four parts. Finally, the ceacum was isolated. The intestinal content of the 10 isolated parts was saved in separate files. Intestinal content was centrifuged (2600 rpm for 20 min) and dye concentration in 75 μl of the supernatant + 1 ml 0.1 M NaOH was measured in a photo-spectrometer (E580). White bars represent RLA rats, Black bars represent RHA rats. From the data the percentage of the total fraction found in each part was calculated. Differences between the RLA and the RHA rats were assessed by repeated measures ANOVA (strain = between subjects factor and the part of the intestine = within subjects factor), followed by multivariate ANOVA post-hoc analysis for the separate intestinal parts. * indicates a significant difference between RLA and RHA rats (RM-ANOVA F $(23,137)$ = 5.623 $p < 0.05$).

circadian rhythmicity meal patterns, in both the Roman and the WTG strain (Fig. 6) and confirmed Rossi's findings in the Roman rat strain, but failed to find differences in circadian eating patterns in the WTGs. It is therefore not yet clear whether the differences in the biological clock in the passive and proactive Romans might be a result of a
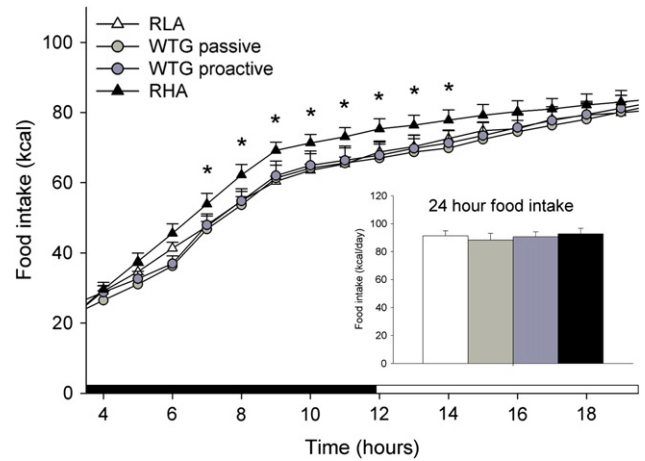

Fig. 6. Cumulative food intake in passive and proactive individuals of the Roman and the Wild Type Groningen rat strains. Food intake was measured continuously using the TSE drinking and feeding monitor system (TSE, Bad Homburg). The figure displays the average intake per day over a period of three weeks. White symbols represent RLA rats (n = 8), Light grey symbols represent passive WTG rats (n = 8), dark grey symbols represent proactive WTG rats (n = 8), and black symbols indicate RHA rats (n = 8). Differences between the experimental groups were calculated using repeated measures ANOVA (experimental group = between subjects factor and the time of day = within subjects factor). The RM-ANOVA was followed by a multivariate ANOVA post-hoc analysis to allow comparison at the different time points. * indicates a significant difference between RHA rats and all other tested rats ($p < 0.01$) (unpublished data from the experiment described in [17]).

Table 5
Coping styles and meal pattern analysis. Meal patterns were assessed using the TSE drinking and feeding monitor system (TSE, Bad Homburg). The meal size was measured with 0.1 g precision. Meal duration was measured with 30 s precision. An arrow indicates a significant difference from controls, "ns" no differences to controls (unpublished data from the experiment described in Ref. [17]).

	RLA	RHA	WTGp	WTGa
Meal size (kcal/meal)	↓	ns	ns	ns
Meal number	↑	ns	ns	ns
Eating rate (kcal/min)	↓	↑	↓	↑
Time with food (min)	ns	ns	ns	ns
Intake in a novel environment	↓	ns	no data	no data
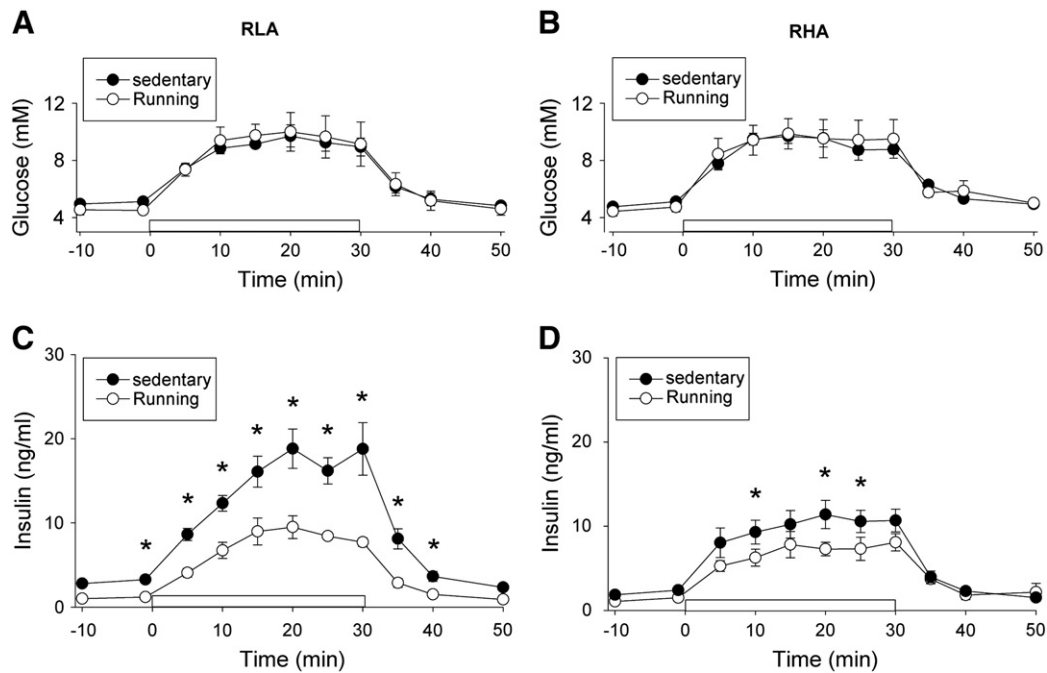
Fig. 7. Glucose and insulin responses to an intravenous glucose tolerance test (IVGTT) in sedentary and running rats of the Roman stain on a chow diet. A: the glucose response to a 30 min glucose infusion (15 mg/ml) in RLA rats (n = 8) on a chow diet. B: the glucose response to a glucose infusion in RHA rats (n = 8) on a chow diet C: the insulin response to a glucose infusion in RLA rats (n = 8) on a chow diet. D: the insulin response to a glucose infusion in RHA rats (n = 8) on a chow diet. The black symbols represent baseline sedentary conditions and the white symbols represent voluntary running conditions. Differences between the RLA and RHA rats were assessed by calculating the area under the response curve followed by repeated measures ANOVA analysis (strain = between subjects factor, condition = within subjects factor). The RM-ANOVA was followed by a multivariate ANOVA post-hoc analysis to allow comparison at the different time points. * indicates a significant difference between the running and the sedentary condition (F [1,28] = 5.435, p < 0.05). [50].

8. Coping style and (spontaneous) physical activity

It is well known that there are marked differences in the locomotor activity between proactive and passive animals (reviewed in Ref. [17]). By definition, proactive animals are active and passive animals are inactive, particularly in threatening or potential stressful environments. This is of course the case for the defensive bury test, the selection procedure that is used in our lab to distinguish between passive and proactive individuals of the WTG strain. But proactive animals are also more active in behavioral tests such as the open field test, the Porsolt forced swim procedure and the elevated plus maze test [47–49].

We tried to replicate these findings and placed passive and proactive Romans in a novel cage with a running wheel and scored their wheel running activity and their home cage ambulatory activity (measured with infra red activity sensors). As expected, proactive rats showed more exploratory behavior and wheel running in the first hours after the animals were moved into the new environment (unpublished data). However, after the rats became habituated to their environment, the passive individuals dramatically increased their running wheel activity to a level that was significantly higher than that of the proactive animals [50]. We found that, after a while, both daily running wheel activity and home cage activity are significantly elevated in the rats with a passive coping style. We also found that, when placed on a palatable high fat diet, only passive but not proactive individuals from both rat strains increase their running activity to counteract the increased energy intake (data not shown).

The most interesting findings, however, are presented in Fig. 7 and Table 6. Fig. 7 gives the blood glucose and plasma insulin profiles during an IVGTT in both sedentary and running RHAs and RLAs. As

mentioned before, the passive Roman Low Avoidance rat is, under baseline conditions, characterized by insulin resistance and increased visceral adiposity. But the RLA rat increases its spontaneous home cage activity and wheel running when it is allowed to run voluntarily in a wheel. All this leads to a normalization of the plasma insulin responses to control (RHA) levels. This phenomenon, increased spontaneous wheel running activity in animals that are normally obese and insulin resistant under sedentary conditions has been observed before in overweight animal models such as the Oleft rats [51] and MC4 knockout mice [52]. Not only the insulin levels

Table 6

Cardiovascular parameters in rest and body composition in sedentary and exercising passive and proactive Roman rats. Before the start of the experiment, the rats in this study were equipped with a transmitter (PA-C40, Data science, St. Paul, MN) to allow cardiovascular measurements. During the whole study the cages of the rats were place on an antenna board (RA1010, Data sciences) to allow constant measurements of cardiovascular parameters. The data were collected and analyses using Dataquest IV (Data sciences) software. Cardiovascular parameters were measured once every 5 min for 10 s. The cardiovascular parameters of the rats were measured for one week (days 0–7) under the standard sedentary conditions. Then the rats were switched to a cage with a running wheel and left to habituate for three weeks. After habituation, cardiovascular parameters were again measured for one week (days 28–35). Differences between the RLA and RHA rats under the difference conditions were assessed with repeated measures ANOVA (strain and condition were between subjects factors).

	RLA sedentary	RHA sedentary	RLA running	RHA running
Heart rate (bpm)	285 ± 5.1	291 ± 6.4	282 ± 2.6	285 ± 3.1
Systolic pressure (Hg/mm)	120 ± 3.5	109 ± 1.3[a]	115 ± 3.5[b]	112 ± 5.9
Diastolic pressure (Hg/mm)	83 ± 2.9	78 ± 2.4	79 ± 2.0[b]	78 ± 2.3
Heart rate variability	4.9 ± 0.3	5.1 ± 0.5	5.9 ± 0.4[b]	5.2 ± 0.9
% Visceral fat	37 ± 2.2	33 ± 1.6[a]	34 ± 1.8[b]	31 ± 2.6

[a] Indicates a significant difference between the RHAs and RLAs within the same condition (p < 0.05) [50].

[b] Indicates significance between the running and sedentary condition in the same animals (RM-ANOVA; p < 0.05).

genetic co-selection within the strain rather than being secondary to differences in coping style.

improved in the RLA rat, but also the increased spontaneous activity normalized visceral adiposity and elevated the systolic and diastolic blood pressures in the resting state (Table 6). Finally, the increased spontaneous activity significantly increased heart rate variability, an indication of increased physical fitness [53] in the RLAs.

To summarize, in a previous paragraph we concluded that the RLA rat could be considered as a non-obese animal model for the metabolic syndrome. The data above reveal that all the symptoms of the metabolic syndrome (insulin resistance, hypertension, and visceral adiposity) in the RLA disappear when the animals are allowed to run voluntarily in a wheel. One should note that the so-called passive RLA rat ran even significantly more than their active counterparts when allowed to run. This suggests that the RLAs used a behavioral strategy (increased physical activity) to compensate for their pathological characteristics in the sedentary state. One may also argue that the standard sedentary state is in fact a pathological condition for laboratory animals [54], particularly those with a passive personality since they are highly influenced by the environmental conditions [13].

9. Personality and metabolism: relevance for humans application

The present studies revealed that there are significant behavioral and (patho)physiological differences between rats with passive or proactive coping styles. The crucial question is: what is the relevance for the human population? Can we simply translate our data to humans with a proactive or passive personality? Unfortunately, available data in humans on the interactions between personality and the metabolic syndrome or the risk to develop insulin resistance are scarce and confusing. This confusion may be partly due to the fact that in most human studies, individual differences in behavior are categorized by means of self rate personality scales. Unfortunately, these scales are subjective because the personality itself may influence the rating process. The differences in methodology of personality/coping style assessment make a direct translation of the data in rodents to the human complex, however, using the physiological and behavioral parameters characteristic of the different personality types could prove helpful. Most studies using physiological and behavioral parameters to identify different personalities found that the typical characteristics of a passive personality, such as increased HPA-axis activity [55,56] and increased anxiety traits [57,58], are indeed associated with an increased risk to develop of insulin resistance. This was confirmed in a few studies using questionnaires to identify the personality of an individual [4,5]. However, there are also several questionnaire studies that report the opposite: an increased risk for the proactive personality [59–61]. It should be mentioned that, although questionnaire studies are the most abundant in the personality literature, they might not be the most objective for a pathophysiological characterization of patients at risk.

Available data in the human literature suggest that particularly the proactive personality has an increased risk in cardiac infarction [62–64]. This is hypothesized to be the result of an interaction between the increased sympathetic stress response and increased exposure to interpersonal stressors in these individuals [65,66]. Consisted with the human data, rats used for hypertension research such as the Spontaneous Hypertensive Rat, were found to be more aggressive and more explorative in anxiety tests compared to less proactive normotensive Wistar control rats [67,68]. Although this is seemingly in contrast with our current findings in the Roman rat strain, one should realize that there are several cardiovascular pathologies in humans, such as arteriosclerosis and stroke, that are certainly not correlated with a proactive personality (reviewed in Ref. [65]). Most of these pathologies are directly associated with increased adiposity which may indicate that, when studying the relation between personality and cardiovascular risk factors, one

should dissociate these risk factors in a group that are related to increased sympathetic activity and a group that are the result of increased adiposity. A proactive personality may be more prone to the first group of pathologies, whereas the passive personalities might be particularly sensitive to the latter group.

In our studies, all symptoms of the metabolic syndrome (insulin resistance, hypertension, and visceral adiposity) disappeared when the RLAs were allowed to run voluntarily in a wheel. We also found that only the passive but not the proactive individuals from both rat strains increase their running activity when they were fed a palatable high fat diet. This means that in particular passive rats are sensitive for potential environmental cues. Human data are more or less similar. Passive personalities are more sensitive to external motivation and external motivation improves performance only in passive but not in proactive personalities [69]. This is an important issue because this differential response to external motivation may be crucial for the development of tailor-made treatment programs. We speculate that particularly the passive personality might be successful in following a lifestyle intervention program, for example for the prevention or treatment of overweight and type 2 diabetes. Proactive personalities, who are much less sensitive to external cues, will probably fail in a lifestyle intervention program and should focus on more intrinsic methods such as pharmacological treatment. Preliminary data from our current studies seem to confirm this.

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