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## The passive coping Roman Low Avoidance rat, a non-obese rat model for insulin resistance

G.J. Boersma<sup>a,\*</sup>, A.J.W. Scheurink<sup>a</sup>, P.Y. Wielinga<sup>a</sup>, T.J. Steimer<sup>b</sup>, L. Benthem<sup>c</sup>

<sup>a</sup> Department of Neuroendocrinology, University of Groningen, The Netherlands

<sup>b</sup> Clinical Psychopharmacology Unit (APSI), University of Geneva Hospital, Switzerland

<sup>c</sup> Bioscience Diabetes/Obesity, AstraZeneca, Mölndal, Sweden

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### ABSTRACT

The aim of the study was develop to an animal model that links coping style to insulin resistance. We hypothesized that the psychogenetically selected Roman Low Avoidance (RLA) rats may serve as such a model. To test this hypothesis, we submitted both RLA and Roman High avoidance (RHA) rats to a series of intravenous glucose tolerance tests (IVGTT). These IVGTT were followed by post mortem metabolic characterization of the selection lines. It was found that plasma insulin levels are markedly elevated in the passively coping RLA rat, both in baseline conditions and during the intravenous glucose tolerance tests. The elevation in plasma insulin was accompanied with increased levels of plasma corticosterone, FFA, leptin and triglycerides but not by changes in body weight. We conclude that the passive, highly emotional RLA rat is metabolically different from both the RHA rat and the standard control Wistar rat and may serve as a non-obese animal model for insulin resistance.

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### 1. Introduction

Most animal models for the metabolic syndrome, insulin resistance, and type 2 diabetes are based upon an obese phenotype. This is consistent with the idea that obesity is one of the major risk factors for the development of these metabolic disorders. A significant proportion of type 2 diabetes patients, however, never suffered from overt overweight during the development of the disease.

Psychosocial stress factors, such as a low level of education, a low sense of coherence, work stress and sleep disorders are also associated with the development of the metabolic syndrome and insulin resistance [1]. Personality plays a role as well, as indicated by the observation in children [2] and adults [3] that a type A personality has a lower risk to develop the metabolic syndrome. However, animal studies on this topic are scarce. To our knowledge, there is no animal study that links psychosocial factors or personality to the development of insulin resistance or the metabolic syndrome. In human studies, patterns of individual characteristics have been generally grouped into types, temperaments or personalities. In rat studies, we use the term coping style to refer to a similar distribution.

Studies with the Roman Low and High Avoidance rat selection lines may fill in this gap. Roman Low and High Avoidance rats (RLA and

RHA, respectively) were originally selected and bred (from a Wistar stock) for rapid versus poor acquisition of a two-way, active avoidance response in the shuttle-box. The poor performance of the RLA rats was later shown to be due to their increased emotional responsiveness, in particular their innate tendency to freeze when confronted with a challenging situation [4]. The behavioral and physiological characteristics of these selection lines are well documented (*for a review, see [4]*). In short, Roman Low Avoidance and Roman High Avoidance rats differ in emotional reactivity and coping style. RLA rats are highly emotional individuals with a *passive* coping style, whereas RHA rats behave as *active* individuals with low emotional reactivity. Experimentally, emotionality can be defined as a reaction to environmental changes characterized by at least two of the following behavioral parameters: decrease exploratory activity in a novel environment, increased duration of the freezing response, shorter latency to self-grooming, or increased defecation [4]. These behavioral responses are also associated with enhanced HPA-axis reactivity, e.g. corticosterone secretion [5]. RHA rats are impulsive and show high levels of novelty seeking behavior [6]. Furthermore, they display rigid behavioral patterns and they have a greater preference for rewarding substances as compared to the RLAs [7]. These differences in coping style are accompanied with differences in several neuroendocrine and metabolic parameters. Most studies focus on neurotransmitters such as dopamine, serotonin and vasopressin [8,9]. But it was also found that the RLA rat is characterized by an increased sensitivity of the hypothalamus-pituitary-adrenal (HPA) axis, leading to increased corticosterone and corticotrophin (ACTH) secretion [5,10,11].

\* Corresponding author. Department of Neuroendocrinology, University of Groningen, PO box 14, 9750 AA Haren, The Netherlands. Tel.: +31 50 363 2345; fax: +31 50 363 2331.

E-mail address: [g.j.boersma@rug.nl](mailto:g.j.boersma@rug.nl) (G.J. Boersma).

Furthermore, it was reported that the RLA rat gained more weight on a high fat diet than the RHA rat and that there were differences in meal patterns between the selection lines [12].

Increased HPA-axis activity and enhanced weight gain on a high fat diet generally correlate with changes insulin sensitivity [13–18]. We therefore hypothesized that the Roman Low and High selection lines might also be metabolically different, particularly in the regulation of insulin release and glucose homeostasis. To test this hypothesis, we submitted both RLA and RHA rats to a series of *in vivo* intravenous glucose tolerance tests (IVGTT) to study the glucose and insulin responses. These IVGTT were followed by a post mortem metabolic characterization of the selection lines. The data revealed that the passive, high emotional RLA rat is metabolically different from both the RHA rat and the standard control Wistar rat and may serve as a (non-obese) animal model for insulin resistance.

## 2. Materials and methods

### 2.1. Animals

Male Roman High Avoidance rats and Roman Low Avoidance rats, weighing 250–300 g at the beginning of the study, were used. The animals were obtained from a breeding colony at the Clinical Psychopharmacology Unit (APSI), University of Geneva, Switzerland. Before the experiments started rats were given sufficient time (at least 3 weeks) for acclimatization. The animals were individually housed in standard cages (24×24×36 cm), lab chow (*Hope Farms, RMH-B knaagdier korrel, Arie Block Diervoeding, Woerden, NL*) and water was available *ad lib*. The room was controlled for temperature and humidity ( $T=20\pm 2$  °C, humidity 60%) and was kept on a 12–12 h light–dark cycle (lights on = CT0, lights off = CT12). All animal experiments were approved by the local animal care committee.

### 2.2. Experimental design

The study consisted of a series of experiments, which started with a defensive bury test to define the coping style of the individual rats. Then the rats underwent surgery to place indwelling jugular vein catheters allowing continuous blood sampling in freely moving animals. After recovery, baseline measurements of food and water intake and body weight were taken for four weeks. Thereafter two intravenous glucose tolerance tests (IVGTT) with different doses of glucose were performed at a two weeks interval. The animals were sacrificed for post mortem carcass and hormone analysis two weeks after the last IVGTT.

### 2.3. Defensive burying test

A defensive bury test was performed to verify the coping style of the rats. The defensive burying test is a coping style test that is independent of the active avoidance selection paradigm. The procedure was first described by Pinel and Treit [17]. The experimental animals were housed in specialized defensive burying cages (24×24×36 cm) with a hole of approximately 1 cm diameter. Through this hole an electric prod can be inserted (shock of 20 mA). After a habituation period of at least a week the animals were tested in the middle of the light phase (CT4–CT10). The electric prod is inserted into the cage and after the first shock the behavior of the rat was monitored for 10 min (Eline software program). The time spent on exploration of the cage, self-grooming, exploration of the prod, burying the prod and immobile (freezing) behavior was scored.

### 2.4. Surgery

All animals were equipped with a double jugular vein catheter. Rats were sedated using an isoflurane-O<sub>2</sub>/N<sub>2</sub>O gas anesthesia. A

silicon heart catheter (0.95 mm OD, 0.50 mm ID, and 0.64 mm OD, 0.28 ID) was inserted into the right jugular vein and kept in place with a ligament. The catheter was pulled under the skin towards the skull where it was connected to a metal bow. This metal bow was fixed to the skull with dental cement and 4 small screws. The same procedure was repeated on the left side. The animals were given 0.1 ml Finadine s.c. for analgesia and 0.25 ml penicillin s.c. to prevent infection. After surgery the rats were allowed to recover for at least 7 days. During blood sampling or infusions a piece of tubing could be attached to the metal bow, hereby samples could be taken from conscious rats. In between experiments, the catheter was filled with a PVP/heparin solution preventing blood clot formation in the catheter [19].

### 2.5. Intravenous glucose tolerance test

Rats were accustomed to the infusion and blood sample procedure before the actual onset of the experiments following a standard procedure described by Steffens [20]. Then, two intravenous glucose tolerance tests (IVGTT) were performed. During the first IVGTT, the rats were infused with 10 mg/min glucose for 20 min through the catheter in the left jugular vein, which is a physiological dose that mimics the glucose response after a meal [21]. The rats were denied access to their food from the beginning of the light phase until the end of the IVGTT; food was removed at CT0. The experiments were performed in the middle of the light phase, between CT4 and CT6. Two baseline blood samples were taken before the start of the infusion ( $t=-15$  and  $t=-5$ ). During and after infusion (from  $t=0$  to  $t=20$  min) blood samples of 0.2 ml were taken at 1, 3, 5, 7, 10, 15, 20, 23, 26, 30 and 40 min. A total volume of 2.8 ml blood was taken and the loss of volume was substituted by saline infusion. During the second IVGTT the rats were infused with 16 mg/ml glucose for 30 min, which is a higher dose to evoke a larger insulin response, but the levels still remain within a physiological range [22]. In this experiment, blood samples were taken at baseline and at 5, 10, 15, 20, 25, 30, 35, 40, and 50 min after the start of the infusion.

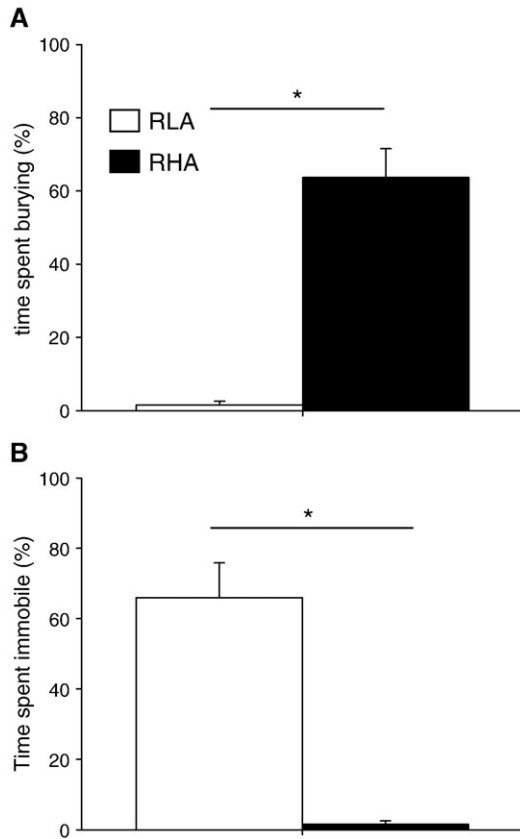
Blood samples were kept on ice and stored in tubes with 10  $\mu$ l EDTA (0.09 g/ml). For glucose determination 50  $\mu$ l of blood with 450  $\mu$ l heparin solution (2%) was stored at  $-20$  °C. The remaining blood was centrifuged for 15 min and plasma was stored for insulin determination.

### 2.6. Post mortem analysis

The rats were sacrificed two weeks after the last IVGTT. Three hours before lights off, blood samples were taken directly from the heart under isoflurane-O<sub>2</sub>/N<sub>2</sub>O gas anesthesia for determination of blood glucose, plasma insulin, and leptin levels. Animals were hereafter sacrificed using an overdose of pentobarbital. Epididymal and retroperitoneal fat pads and the liver were taken out and weighed. Hereafter, biopsies were taken from the liver (*left ventral lobe*) for further analysis. 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 Soxhlet 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.

### 2.7. Plasma fuel and hormone analysis

Plasma levels of insulin and leptin were measured using commercial radioimmunoassay (RIA) kits (*Linco Research*). Plasma corticosterone level were determined with a commercial RIA kit (*MP Biomedicals*). Plasma non-esterified fatty acids, plasma cholesterol levels, and liver triglyceride levels were measured using commercial



**Fig. 1.** A: Percentage time spent burying for RLA ( $n = 10$ , white bars) and RHA ( $n = 10$ , black bars) rats in the defensive burying test ( $F_{1,15} = 50.276$ ,  $P < 0.01$ ). B: Percentage time spent immobile for RLA and RHA rats in the defensive burying test. \* indicates a significant difference from RLA rats ( $F_{1,15} = 47.266$ ,  $P < 0.01$ ).

kits (Astra Zeneca). Blood glucose levels were determined using the ferricyanide method (Hoffman, 1937) in an auto analyzer (Technicon).

### 2.8. Statistical analysis

The data are expressed as averages with standard error of the mean. Differences in food and water intake, body weight, the defensive burying test, and baseline plasma levels between selection lines were determined using a one-way ANOVA. The selection line was the between subjects factor. Differences in insulin and glucose levels before, during, and after the IVGTT were examined using a repeated measures ANOVA. The selection line was the between subjects factor. The area under the curves of both glucose and insulin responses were calculated and reported as the average area under the curve (AUC) with the standard error of the mean. The differences between the

**Table 1**

Percentage of time spent on other behaviors than burying or immobility during the defensive burying test of RLA ( $n = 8$ ) and RHA rats ( $n = 8$ ).

	RLA	RHA
Immobile	65.0 ± 9.9	1.5 ± 1.0 <sup>a</sup>
Bury prod	1.5 ± 0.9	63.7 ± 7.8 <sup>a</sup>
Explore cage	8.0 ± 3.2	16.1 ± 6.2
Explore Prod	12.7 ± 10.2	13.3 ± 4.1
Grooming	12.4 ± 4.0	9.0 ± 4.0

<sup>a</sup> Indicates a significant difference Bury prod ( $F_{1,15} = 50.276$ ,  $P < 0.01$ ), Immobile ( $F_{1,15} = 47.266$ ,  $P < 0.01$ ).

**Table 2**

Baseline body weight, food intake and water intake of RLA ( $n = 8$ ) and RHA rats ( $n = 8$ ).

	RLA	RHA
Body weight (g)	410.5 ± 12.5	391.8 ± 9.6
Food intake (kcal/day)	70.8 ± 4.3	67.9 ± 3.4
Water intake (g/day)	40.7 ± 1.49	33.6 ± 1.52 <sup>a</sup>

<sup>a</sup> Indicates a significant difference ( $F_{1,15} = 6.307$ ,  $P < 0.05$ ).

selection lines were determined using a one-way ANOVA. A confidence interval of 5% was used.

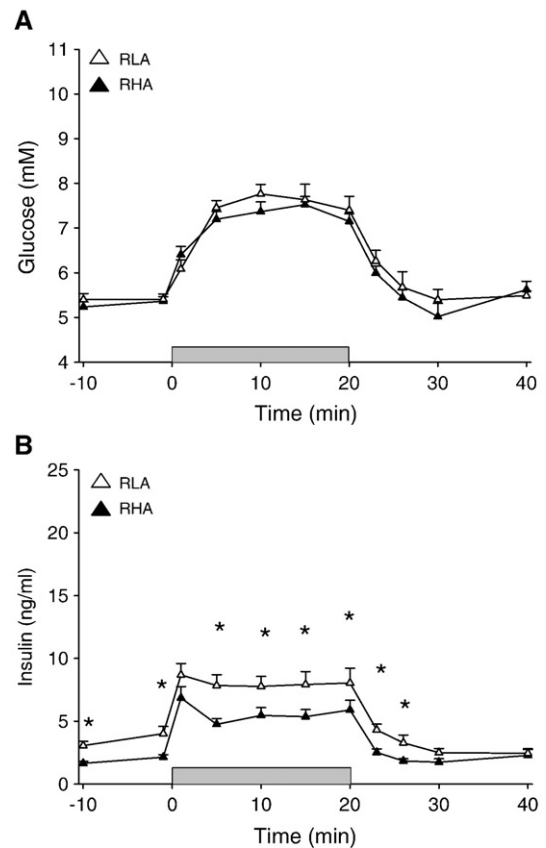
## 3. Results

### 3.1. Defensive burying test

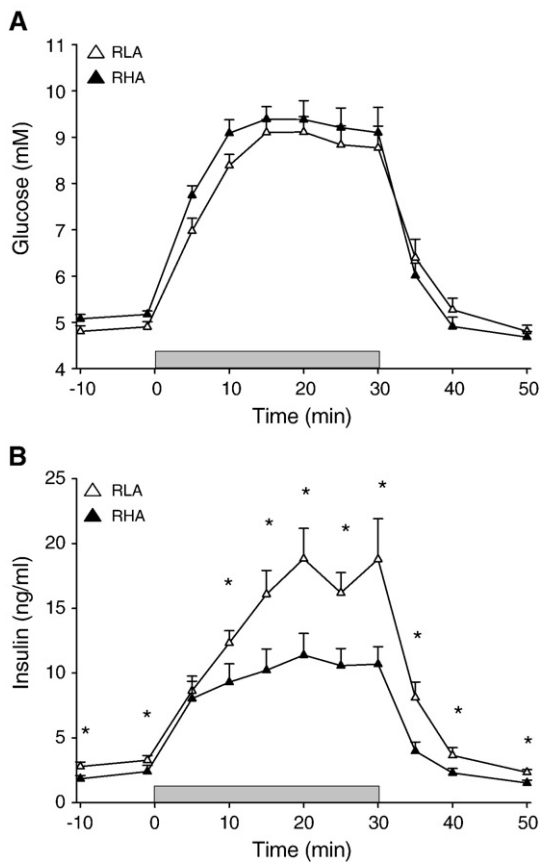
Fig. 1 shows the behavior of the rats in the defensive burying test during the first 10 min after receiving a shock. RHA rats spent significantly more time burying the prod ( $F_{1,15} = 50.276$ ,  $P < 0.01$ ) and displayed significantly less immobility behavior than RLA rats ( $F_{1,15} = 47.266$ ,  $P < 0.01$ ) (Fig. 1). There were no differences in other behaviors than burying and immobility (Table 1).

### 3.2. Body weight, food intake, and water intake

Table 2 displays the body weight, the food intake, and water intake during the four weeks baseline measurements. There were no differences in body weight or weight gain between RLAs and RHAs in the four week baseline period. Food intake was not different



**Fig. 2.** A: Glucose response of RLA ( $n = 12$ , open symbols) and RHA ( $n = 12$ , closed symbol) rats during an intravenous glucose tolerance test. B: Insulin response of RLA and RHA rats during an intravenous glucose tolerance test. Grey bar indicates infusion of a 10 mg/mg glucose. \* indicates a significant difference ( $F_{1,15} = 3.062$ ,  $P < 0.01$ ).



**Fig. 3.** A: Glucose response of RLA ( $n = 10$ , open symbols) and RHA ( $n = 10$ , closed symbol) rats during an intravenous glucose tolerance test. B: Insulin response of RLA and RHA rats during an intravenous glucose tolerance test. Grey bar indicates infusion of a 16 mg/min glucose. \* indicates a significant difference from the RLA rats ( $F_{1,15} = 3.973$ ,  $P < 0.01$ ).

between the two selection lines. Water intake was significantly higher in RLAs compared with RHAs ( $F_{1,15} = 6.307$ ,  $P < 0.05$ ).

### 3.3. IVGTT

Figs. 2A and 3A reveal the blood glucose levels during the 10 and 16 mg/min IVGTT respectively. Blood glucose levels started at a baseline of approximately 5 mM in both conditions and increased to a maximum of 7.8 mM (10 mg/min) and 9.6 mM (16 mg/min) during infusion. After termination of the infusion, blood glucose levels returned towards baseline levels within 10 min. In both experiments there were no significant differences between RLA and RHA rats.

Figs. 2B and 3B display the plasma insulin levels during the 10 and 16 mg/min IVGTT respectively. In both experiments, baseline plasma insulin levels were significantly higher in RLAs in comparison to the RHAs. This difference in insulin levels between the RLAs and RHAs remained significant throughout both IVGTT ( $P < 0.01$ ).

Glucose and insulin responses were also calculated as area under the curve (AUC), which are presented in Table 3. The AUCs for glucose

**Table 3**

Area under the curve during an IVGTT in RLA ( $n = 10$ ) and RHA rats ( $n = 10$ ) corrected for baseline values.

	RLA	RHA
10% Glucose	130.4 ± 14.0	119.4 ± 5.0
10% Insulin	123.5 ± 25.9	101.4 ± 22.3
16% Glucose	197.1 ± 16.6	196.3 ± 18.8
16% Insulin	344.5 ± 36.6	289.3 ± 27.7 <sup>a</sup>

<sup>a</sup> Indicates a significant difference ( $F_{1,19} = 9.402$ ,  $P < 0.01$ ).

**Table 4**

Baseline values of blood parameters of RLA ( $n = 8$ ) and RHA rats ( $n = 8$ ).

	RLA	RHA
Corticosterone (ng/ml)	364.4 ± 42.1	228.5 ± 30.9 <sup>a</sup>
Leptin (ng/ml)	4.46 ± 0.64	3.19 ± 0.76 <sup>a</sup>
Non-esterified fatty acids (mM)	0.23 ± 0.04	0.19 ± 0.3
Liver triglycerides (g/100 g tissue)	3.5 ± 0.3	2.3 ± 0.3 <sup>a</sup>
Total cholesterol (mM)	1.54 ± 0.17	1.11 ± 0.12 <sup>a</sup>

<sup>a</sup> Indicates a significant difference. Corticosterone ( $F_{1,13} = 6.989$ ,  $P < 0.01$ ), Leptin ( $F_{1,13} = 3.156$ ,  $P < 0.05$ ), total cholesterol ( $F_{3,11} = 2.552$ ,  $P < 0.05$ ), liver triglycerides ( $F_{3,11} = 3.214$ ,  $P < 0.01$ ).

were not different between the RLAs and the RHAs. The AUC for insulin was significantly higher in the RLAs compared to the RHAs during the 16 mg/min glucose infusion ( $F_{1,19} = 9.402$ ,  $P < 0.01$ ), the differences in insulin response during the 10 mg/min infusion just failed to reach significance ( $P = 0.06$ ).

### 3.4. Post mortem analysis

Table 4 displays the post mortem plasma levels of corticosterone, leptin, non-esterified fatty acids (NEFA), total cholesterol, triglycerides, and the body composition of the RLA and RHA rats. Plasma levels of corticosterone ( $F_{1,13} = 6.989$ ,  $P < 0.01$ ), leptin ( $F_{1,13} = 3.156$ ,  $P < 0.05$ ) total cholesterol ( $F_{3,11} = 2.552$ ,  $P < 0.05$ ), and levels of triglyceride in the liver ( $F_{3,11} = 3.214$ ,  $P < 0.01$ ) were significantly higher in RLA rats than in their RHA counterparts. No significant differences were found in NEFA levels.

Table 5 presents the fat distribution as determined by carcass analysis. The RLA rats had a significantly higher epididymal fat weight than RHA rats ( $F_{1,13} = 7.564$ ,  $P < 0.05$ ). There were no significant differences in lean body mass, total fat percentage and retroperitoneal and subcutaneous fat weight. The results remained the same when the data were corrected for lean body mass or total fat mass.

## 4. Discussion

In this study we characterized the metabolic profiles of Roman High and Low Avoidance selection lines. RHA and RLA rats were originally selected and bred for a rapid versus poor acquisition of the active avoidance response. They differ in emotional reactivity and coping style. RLA rats are highly emotional individuals with a passive coping style, whereas RHA rats behave as active individuals with low emotional reactivity. We hypothesized that due to the increased HPA-axis activity displayed in the RLA rats these animals could be prone for the development of the metabolic syndrome.

We found that indeed the passive RLA coping style was associated with insulin resistance and elevated levels of plasma leptin, FFAs, liver triglycerides, and an increased visceral fat content. The pro-active RHA rat revealed no signs of adiposity or insulin resistance. In fact, the glucose and insulin profiles in RHA rats were remarkably similar to those that were found in numerous previous studies in Wistar rats in our laboratory throughout the years [21,23,24]. Taken together this

**Table 5**

Carcass analysis of RLA ( $n = 8$ ) and RHA rats ( $n = 8$ ).

	RLA	RHA
Body mass	435 ± 14	401 ± 16
Epididymal fat (g)	4.95 ± 0.24	4.10 ± 0.34 <sup>a</sup>
Retroperitoneal fat (g)	7.85 ± 1.42	9.79 ± 1.37
Subcutaneous fat (g)	36.92 ± 1.95	37.66 ± 0.87
Total body fat (%)	11.56 ± 0.55	13.09 ± 1.54
Lean carcass weight (g)	306.2 ± 0.4	293.4 ± 11.6
Ratio epididymal/retroperitoneal	0.64 ± 0.03	0.47 ± 0.03 <sup>a</sup>

The table displays wet fat mass. The total body fat was calculated as a percentage of the total body mass at sacrifice.

<sup>a</sup>Indicates a significant difference ( $F_{1,13} = 7.564$ ,  $P < 0.05$ ).

suggests that in particular the highly emotional individual with a passive coping style might have an increased risk for the development of metabolic diseases as insulin resistance and the metabolic syndrome. This means that the Roman Low Avoidance rat may be considered as a non-obese rat model for insulin resistance.

Plasma corticosterone levels were significantly elevated in the RLA rats. This confirms previous findings in the literature, in which it was shown that RLA rats have an increased sensitivity of the hypothalamus-pituitary-adrenal (HPA) axis, leading to increased corticosterone and corticotrophin (ACTH) secretion [10,11].

Increased HPA-axis activity has been reported to be a potent mediator of insulin resistance [16,25–27]. Patients with Cushing's syndrome, with an excess of corticosteroids, commonly express severe insulin resistance [28]. Moreover, humans [29] as well as animals [16,30] treated with (synthetic) glucocorticoids develop insulin resistance. One should note that the phenotype of dexamethasone-induced insulin resistant rats does not involve an increase in body mass [31] which is similar to what we observed in the present study.

The mechanism behind glucocorticoid-induced insulin resistance has not been fully elucidated, however glucocorticoid-induced insulin resistance is generally associated with an impairment of insulin's actions to suppress hepatic glucose production and to stimulate glucose utilization [28,32]. Additionally, glucocorticoids seem to have a direct inhibitory effect on glucose-induced insulin release in the  $\beta$ -cells. Corticosteroids have been suggested to induce insulin resistance via an increase in circulating FFAs. However, dexamethasone-induced impairment in skeletal muscle glucose transport is not reversed by inhibition of FFA oxidation [33], indicating that corticosteroids may have a direct effect on insulin sensitivity. This is confirmed in an animal model for high fat feeding-induced insulin resistance in skeletal muscle, where treatment with the anti-glucocorticoid RU-486 resulted in an amelioration of insulin resistance [34].

The RLA rats display a differential fat distribution, favoring visceral fat. Carcass analysis showed a small but significant difference in body composition: epididymal adiposity was higher in RLA rats than in RHA rats. There are numerous studies that suggest that there is a direct relation between the amount of visceral adiposity and the severity of insulin resistance (reviewed in [13]). Likewise, removal of visceral fat in obese hepatic insulin resistant rats reverses insulin resistance [25]. The observed increased epididymal fat mass might therefore have influenced the insulin levels in the RLA rats (or vice versa). The underlying mechanism remains under debate, however, there is evidence that increased HPA-axis activity plays a part.

Olefsky et al. [35] have shown that the visceral adiposities display higher densities of glucocorticoid receptors than adiposities from the subcutaneous depots. This has led to the hypothesis that a fat distribution favoring visceral adiposities in combination with elevated levels glucocorticoids may have exacerbate the shift of lipids to the skeletal muscles [36]. In turn, elevated lipids levels in the skeletal muscles are associated with increased insulin resistance [37]. Interestingly, increased portal venous supply of long-chain fatty acids from the visceral fat depots to the liver induces HPA-axis activation, thus amplifying this process [26]. Based on these data, we might conclude that in the RLA rats the increased epididymal fat depot in interplay with high levels of glucocorticoids may have led to the observed increased in baseline insulin levels and increased insulin resistance.

Plasma levels of leptin were also elevated in the RLA rats, consistent with the differences in insulin but inconsistent with the finding that there are no major differences in fat mass between RLAs and RHAs. This is surprising, and might be explained by a direct effect of elevated insulin levels on leptin production [38,39].

In summary, this study showed that the coping style of an individual is clearly associated with particular metabolic and (patho)physiological characteristics. The highly emotional, passively coping Roman Low Avoidance rats show insulin resistance already at

normal weight. The RLA rat may therefore be considered as a (non-obese) animal model for insulin resistance under standard chow conditions. These standard chow conditions are important since Rossi et al. [12] have already shown that RLA rats are highly susceptible for weight gain on a high fat diet. Our future studies will therefore primarily focus on the effect of the interaction between a passive coping style and changing dietary conditions on the susceptibility for diseases like insulin resistance, type 2 diabetes mellitus, and the metabolic syndrome.

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