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Coping styles and the pathophysiology of energy metabolism

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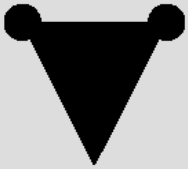
CHAPTER 4:



Coping style predicts the (in)sensitivity for developing hyperinsulinemia on a high fat diet in rats.

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Abstract

The aim of this study was to explore interactions between coping style and diet as risk factors for developing insulin resistance in rats. We hypothesized that rats characterized by a passive coping strategy are more susceptible for developing insulin resistance and visceral obesity than proactively coping rats, particularly on a high (45%) fat diet. This hypothesis was tested by comparing 1) insulin and glucose responses to an intravenous glucose tolerance test (IVGTT), and 2) body fat distribution, in two rat models for passive and proactive coping styles. We found that the most extremely passive rats are characterized by elevated insulin levels during a IVGTT, even on chow. Moderately passive rats display normal insulin responses under chow conditions, but develop insulin resistance on a high fat diet. Proactive rats are remarkably resistant to insulin resistance and visceral obesity, even when overfeeding on a high fat diet. Carcass analysis revealed that passive rats are characterized by increased epididymal fat deposition, which is in line with the observed differences in insulin resistance. We conclude that a passive personality is prone to develop insulin resistance and visceral obesity on a palatable fat diet and a the proactive personality might protected against the development of diet-induced insulin resistance.

Keywords: insulin, glucose, personality, visceral obesity

1. Introduction

The incidence of insulin resistance and type 2 diabetes is rapidly growing. Insulin resistance is characterized by a reduction in the sensitivity of the insulin receptor or post-receptor signaling cascades, which presents itself by increased insulin levels in normo- or hyperglycemic individuals (1). An energy-dense diet is one of the risk factors for development of insulin resistance. Rats fed a high fat (HF) diet usually weigh more than standard laboratory chow (high-fibered carbohydrate-rich) fed rats. HF-feeding rats develop more adipose tissue and acquire insulin resistance (2). But ingestion of a HF diet may also increase fat stores at the expense of fat-free mass and leave body weight unaltered (3). Increases in body fat, especially viscerally stored fat, is associated with insulin resistance, and, additionally, basal plasma insulin levels are directly correlated with the degree of adiposity (4).

Not only the diet of an individual is involved in the development of insulin resistance. Psychosocial factors have been implicated as well. Several studies have shown correlations between certain personality traits of the individual and the incidence of insulin resistance (5;6). Although some discrepancy exists in the literature, individuals with the type B personality may have a higher risk for the development of insulin resistance (7;8). This seems to be in line with our recent data that a rat strain selected for a so-called passive coping style (i.e., which is homologous to the type B personality in humans) is characterized by elevated insulin levels and increased adiposity (9).

In the present study we further explored the interactions between coping style and diet as risk-factors for the development of insulin resistance in rats. We hypothesize that 1) rats with a passive coping style are prone to develop insulin resistance on a diet with a high (saturated) fat content and 2) that animals with a proactive coping style are resistant to develop diet-induced insulin resistance. To study this, we selected passive and proactive individuals from two different rat strains and subjected them either to standard laboratory chow or a highly palatable high fat diet. In all animals, glucose tolerance and insulin responses were assessed with an intravenous glucose tolerance test, and fat storage patterns were measured. The data revealed that passive coping rats are indeed susceptible for developing marked hyperinsulinemia and visceral obesity (determined by epididymal fat deposition) on a palatable fat diet and that the proactive rats are protected against these derangements.

2. Materials and methods

The experiments were based on the following aims: 1) to replicate our previous finding (9) that the extremely passive coping style in the selected Roman Low Avoidance (RLA) rat strain is associated with elevated baseline and IVGTT insulin levels, 2) to investigate whether this is also true for passive individuals from a standard rat population (Wild Type Groningen), 3) to study the interaction between personality and diet on risk factors for Diabetes. The experimental groups are given in table 1, the different procedures are explained below.

Table 1: Experimental groups. RLA = Roman Low Avoidance rat, RHA = Roman High Avoidance rat, WTGp = passive Wild Type Groningen rat, WTGa = proactive Wild Type Groningen rat.

strain	n	diet	IVGTT
RLA	8	Chow	10mg/ml and 15mg/ml
RHA	8	Chow	10mg/ml and 15mg/ml
WTG p	8	Chow	10mg/ml
WTG a	8	Chow	10mg/ml
WTG p	8	High fat	10mg/ml
WTG p	8	High fat	10mg/ml
RLA	8	High fat	15mg/ml
RHA	8	High fat	15mg/ml

2.1. Animals and housing

The studies were performed with male rats from two different strains. Roman High and Low Avoidance rats (16 of each strain, 418 ± 8.5 gram at the onset of the experiments) were obtained from a breeding colony at the Clinical Psychopharmacology Unit (APSI), University of Geneva, Switzerland. The Roman High and Low avoidance rats (RHA and RLA, respectively) were originally selected by Bignami (10) for their performance in a two-way active avoidance test, and a breeding colony of these rats is maintained at the University of Geneva. RHA rats, are characterized by high levels of aggression, rigid behavioral patterns and a proactive approach towards stressors. The RLA rats, are characterized by low aggression levels, flexible behavioral patterns and a passive stress responses(10) . Wild type Groningen rats (n=20, 468 ± 11.8 gram at the onset of the experiments) were derived from the colony at the University of Groningen, the Netherlands. This rat population is characterized by large intra-strain variation in coping behavior, and is originally derived from the Agricultural University of Wageningen, the Netherlands and is currently bred in Groningen under conventional conditions.

All animals were individually housed in standard cages (24x24x36 cm) with a food hopper on the side. The rats were fed either a high fat diet (Hope Farms, RMH-B knaagdier korrel, Arie Block Diervoeding, Woerden, NL; 4.8 kcal/g, 45 % fat), or 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*. The room was controlled for temperature and humidity (T=20℃, humidity 60%) and kept on a 12/12 hours light/dark cycle (Lights on =(CT0), lights off = CT12) All experiments were approved by the local animal care committee (Dier Experimenten Commissie, Groningen, the Netherlands)

2.2. Surgery:

All animals were equipped with two indwelling jugular vein catheters to allow stress free glucose infusion and frequent blood sampling during the intravenous glucose tolerance test (IVGTT). During surgery, the rats were sedated using an isoflurane- O_2/N_2O gas anesthesia. The jugular vein catheters were placed according to the methods described by Steffens (11). The animals were given 0.1 ml Finadine s.c. for analgesia and 0.25 ml penicillin subcutaneously to prevent infection. After surgery the animals were given at least 7 days to recover. Rats were accustomed to the infusion and the blood sample procedure before the actual onset of the experiments (12).

2.3. Defensive bury test:

Two weeks after surgery, the coping style or personality of each animal was assessed with the defensive bury test (first described by Pinel and Treit(13)). In short, the animals were housed in special defensive burying cages (standard rat cages of 24x24x36 cm with a hole of approximately 1 cm diameter). After a habituation period of at least a week the animals were tested. The rats were tested in the middle of the light phase. The electric prod was inserted through the hole in the cage and when the rats touched the prod they received a mild shock (20 mA). After the shock, the behavior of the rat was monitored for 10 minutes (Eline software program). The following behaviors were scored: immobility, exploration of the prod, exploration of the cage and burying of the prod. The percentage time spent burying the prod was the main criterion for the coping style: animals burying 10 or less percent of the time were characterized as passive (WTGp), rats burying 20 or more percent of the time were characterized as proactive, (WTGa) and rats that were between the cut-off criteria (10-20% burying) were excluded from the study (n=4).

2.4. Experiments:

The experimental groups are described in table 1. The animals were fed either chow or the palatable high fat diet for three weeks. The start of the diet was designated day 0. Body weight and food intake was measured daily around CT 4. At day 24-26 an intravenous glucose tolerance test (IVGTT) was performed and blood samples were taken for measurement of blood glucose and plasma insulin levels. An IVGTT consisted of either a 20 minutes intravenous infusion of 10 mg glucose in 0.1 ml saline per minute (total 200 mg glucose in 2 ml saline) or a 30 minutes infusion of 15 mg in 0.1 ml saline per minute (total 450 mg in 3 ml, a relatively high dose of glucose that still remained within the physiological range, (14)).

The protocol for the IVGTT was the following. On the experimental day 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. During IVGTT1 the rats were infused with 10 mg/min glucose over a 20 minutes period. Before the onset of the infusion, two baseline samples were taken at time points t = -11 and -1 minutes. After the start of the infusion at t = 0 minutes, blood samples were taken at time points t = 5, 10, 15 , 20, 25, 30, and 40 minutes. During IVGTT2 the rats were infused with 15 mg/min glucose over a 30 minutes period. Again, two baseline samples were taken at time points t = -11 and -1 minutes. After the start of the infusion (t=0 min) samples were taken at time points t = 5, 10, 15 , 20, 25, 30, 35, 40, and 50 minutes.

Blood samples were kept on ice and stored in files with 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 (Hoffman, 1937(15)) in a Techinicon auto analyzer. The remaining blood was centrifuged for 15 minutes and plasma was stored further analysis. Plasma levels of insulin were measured using commercial radioimmunoassay (RIA) kits (Linco Research).

2.5. Post mortem analysis:

One week after the last IVGTT the rats were sacrificed by decapitation under a light CO_2 anesthesia (day 31-33). After decapitation trunk blood was captured for analysis and all organs were taken out. Epididymal fat pads, retroperitoneal fat pads, and the liver were weighed. Hereafter skin and subcutaneous fat pads were removed from the carcass and were weighed, dried at 80 °C for 5 days, and weighed again. Then the dry tissue was wrapped in paper bags, and the fat content was determined by extracting the fat from the

tissue using a petroleum based Soxlet fat extractor. After fat extraction the paper bags were dried for 5 days and than weighed. The ratio dry weight before/after fat extraction gives an indication of the fat content. Plasma levels of leptin were measured in trunk blood using commercial radioimmunoassay (RIA) kits (Linco Research). Intra- and inter-assay coefficients of variation of reference plasma analyzed in duplicate for the leptin, insulin and corticosteron assays ranged between 6.5-11.3% and 10.3-16.8%, respectively."

2.6. Data analysis:

All data are displayed as an average of the strain with the standard error of the mean. One animal was omitted from the IVGTT based data, because of technical problems during the glucose infusion. Differences in food intake, body weight, water intake and the defensive bury test between the two strains were determined for each diet using a multivariate ANOVA with the diet and the strain as between subjects factors. With this test an interaction effect of diet and strain could also be determined. Differences between the strains and the diets in the insulin and glucose response before, during and after a glucose infusion were tested with a repeated measures ANOVA with strain and diet as between subjects factors. The area under the curves of the 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 difference between the strains was determined using a one-way ANOVA. Strain differences in blood and plasma parameters (glucose, leptin, Insulin) were statistically tested using a one-way ANOVA. Diet-strain interactions were further assessed using Tukey post-hoc analysis. A linear backward regression analysis was performed to analyze the relative contribution of the several measured parameters on the insulin response. In this analysis the area under the insulin curve was used as the dependent factor. Coping style, diet, plasma leptin level, body weight, total fat mass, total fat free mass, retroperitoneal fat mass, subcutaneous fat mass and epididymal fat mass were used as the independent factors. In all statistical tests a confidence interval of 5% was used.

3. Results

3.1. Defensive bury test

Figure 1 shows the burying behavior of the rats in the defensive bury test during the first 10 minutes after receiving a shock. Proactive coping rats, both RHA and WTGa, spent significantly more time burying the prod ($F(3,27) = 50.276$, $P<0.01$) and were less immobile ($F(3,27) = 47.266$, $P<0.01$) than their passive coping counterparts. Proactive RHA rats spent also more time burying than proactive WTG rats ($p<0.05$). On chow, the passive RLA rats tended to bury less than passive WTG rats, this tendency did however not reach statistical significance ($p = 0.085$). There were no significant differences in the time spent on other behaviors than burying and immobility. The diet of the rats did not an effect on the amount of time spent burying, immobility or on any other behaviors between experimental groups.

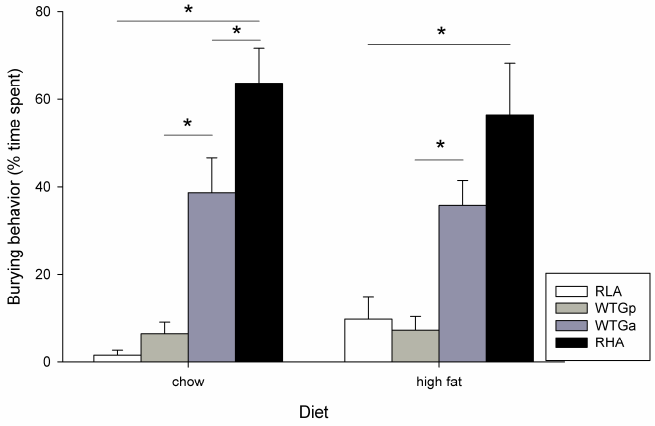

Figure 1: Percentage time spent on bury behavior in a defensive bury test by passive WTG (WTGp) , proactive WTG (WTGa), RLA and RHA rats fed either chow or medium fat diet. Light grey bars represent WTGp rats, dark grey bars represent WTGa rats, white bars represent RLA rats, and black bars represent RHA rats. ** indicates a significant difference with all other experimental groups ($p<0.01$) on the same diet. # indicates a significant difference with RHA and WTGa rats ($p<0.01$) and a trend for a difference with RLA rats ($p<0.1$).

3.2. Body weight and food intake at baseline

Table 2 summarizes the body weight and food intake data. On chow there were no differences between the groups. Both body weight gain and food intake were significantly higher on medium fat diet than on chow ($F(3,27) = 17.246$ $p<0.05$ and $F_{3,27} = 91.375$ $p<0.001$, respectively). On the high fat diet there were also differences between passive and

pro-active personalities: average daily food intake of passive personalities (RLA and WTGp) was significantly higher than that in pro-active rats (RHA and WTGa) ($F(1,30) = 9.305$ $p<0.01$). The differences in weight gain between the coping styles within the RLA/RHA or WTGp/WTGa strains almost reached significance ($p= 0.073$ and $p = 0.094$ respectively).

Table 2: Food intake and body weight data of WTG passive, WTG proactive, RLA and RHA rats. Body weight was measured before the start of the diet intervention. Body weight gain is expressed as % increase per day over a three week period, daily food intake is expressed as average 24 hour food intake over a three week period. * indicates a significant difference from chow fed rats. ^{B} indicates a significant difference between passive and pro-active individuals within the same strain.

	RLA	RHA	WTG passive	WTG proactive
Body weight gain on the diet (%)				
Chow	2.59 ± 0.36	2.41 ± 0.31	2.80 ± 0.57	2.37 ± 0.58
High fat	4.44 ± 0.57*	4.44 ± 0.41*	3.81 ± 0.67*	3.81 ± 0.81*
Daily food intake (Kcal)				
Chow	70.8 ± 4.3	67.9 ± 3.4	72.9 ± 2.8	74.7 ± 3.3
High fat	118.8 ± 4.0*^{B}	109.1 ± 7.3*	116.3 ± 6.2*	106.3 ± 4.7*

3.3. IVGTTs

Figure 2 depicts the glucose and insulin levels before, during and after the IVGTT of all groups on chow (10 mg IVGTT). The glucose levels were not different. The RLA rats displayed a significantly higher insulin level during the IVGTT. The area under the curve for insulin was significantly higher in the RLA rats when compared to those in all other experimental groups ($F(3,28) = 9,368$ $p<0.01$) (Table 3).

Figure 3 shows the plasma glucose and insulin responses of RLA and RHA rats on both chow and high fat diet (15 mg IVGTT). Glucose responses were not different. The area under the curve for insulin responses was significantly higher in the RLAs in comparison to the RHAs, both on chow as well as on high fat ($F(3,25)= 6.609$, $p<0.01$) (Table 4). Insulin was also higher in the RLA rats on high fat diet ($p<0.01$) when compared with RLAs on chow. The areas under the curve for insulin in the RHA rats on chow and high fat were remarkably similar (Table 4).

Figure 4 shows the curves for the glucose and insulin level during an IVGTT of passive and proactive WTG rats on both chow and high fat diet (10 mg IVGTT). Glucose responses were not different. The area under the curve for insulin was significantly higher in the passive WTG rats on the high fat diet when compared to the other WTG groups ($F(1,12) = 8.691$, $p<0.05$) (Table 3).

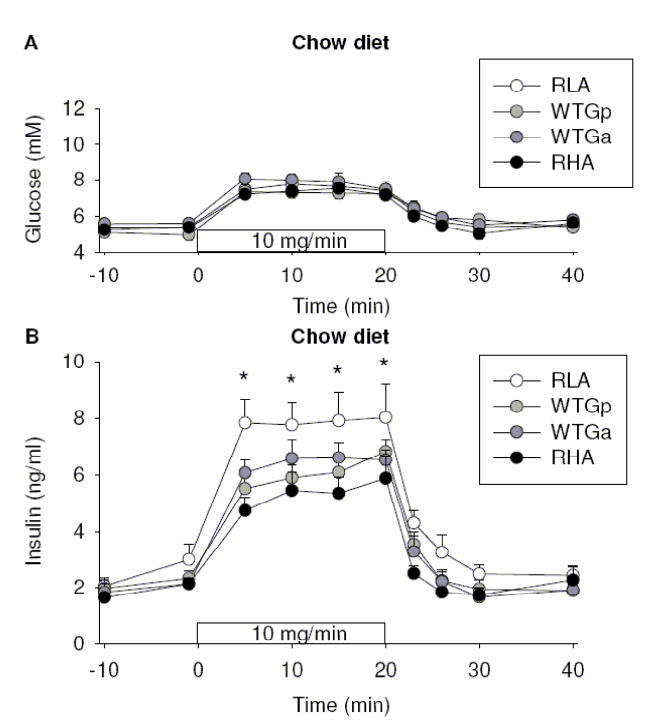

Figure 2: A: Glucose levels before, during and after a 20 minutes glucose infusion for passive WTG (WTGp) , proactive WTG (WTGa), RLA and RHA rats on chow. **B:** Insulin levels before, during and after a 20 minutes glucose infusion for passive WTG (WTGp), proactive WTG (WTGa), RLA and RHA rats on chow. Light grey triangles represent passive WTG rats, dark grey triangles represent RHA rats, white circles represent RLA rats, and black circles represent RHA rats. * indicates a significant difference from WTG passive , WTG proactive and RHA rats ($p<0.01$).

Table 3: Area under the curve for the glucose response and insulin to a 20 minutes glucose infusion for passive WTG (WTGp) , proactive WTG (WTGa), RLA and RHA rats on either a chow or a high fat diet chow. * indicates a significant difference from chow fed rats. [A] indicates a significant difference between RLA rats and all other experimental groups. [B] indicates a significant difference between passive and pro-active individuals within the same strain.

	RLA	RHA	WTG passive	WTG active
Plasma insulin (area under curve)				
Chow	289.5 ± 31.9[A]	196.3 ± 17.5	213.5 ± 9.3	230.2 ± 16.8
High fat			317.6 ± 38.3*[B]	234.6 ± 15.8
Blood glucose (area under curve)				
Chow	297.5 ± 11.6	298.8 ± 4.9	298.3 ± 14.8	298.5 ± 9.8
High fat			301.3 ± 10.4	298.2 ± 14.6

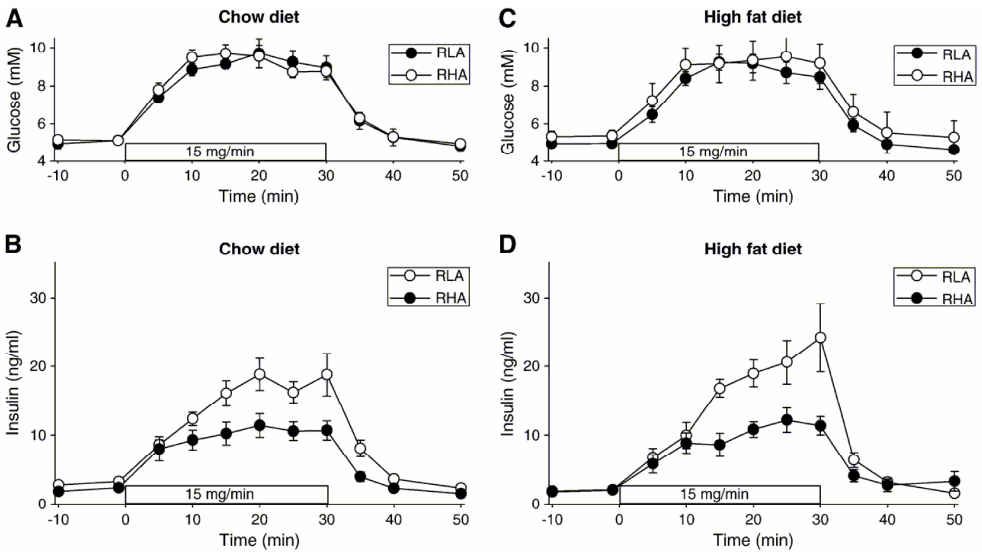

Figure 3: **A:** Glucose levels before, during and after a 30 minutes glucose infusion in Roman high and low avoidance rats fed a chow diet. **B:** Insulin levels before, during, and after a 30 minutes glucose infusion in roman high and low avoidance rats fed a chow diet. **C:** Glucose levels before, during and after a 30 minutes glucose infusion in Roman high and low avoidance rats fed a high fat diet. **D:** Insulin levels before, during, and after a 30 minutes glucose infusion in roman high and low avoidance rats fed a high fat diet. White circles represent RLA rats, black circles represent RHA rats. * indicates a significant difference between RLA and RHA rats ($p<0.01$).

Table 4: Area under the curve for the glucose response and insulin to a 30 minutes glucose infusion RLA and RHA rats on either a chow or a high fat diet chow. * indicates a significant difference from chow fed rats. ^{B} indicates a significant difference between RLA and RHA rats.

	RLA	RHA
Plasma insulin (area under curve)		
Chow	409.3 ± 36.8 ^{B}	304.9 ± 24.4
High fat	501.1 ± 50.3*^{B}	300.2 ± 45.2
Blood glucose (area under curve)		
Chow	313.6 ± 11.6	312.3 ± 16.5
High fat	330.5 ± 14.7	344.7 ± 41.1

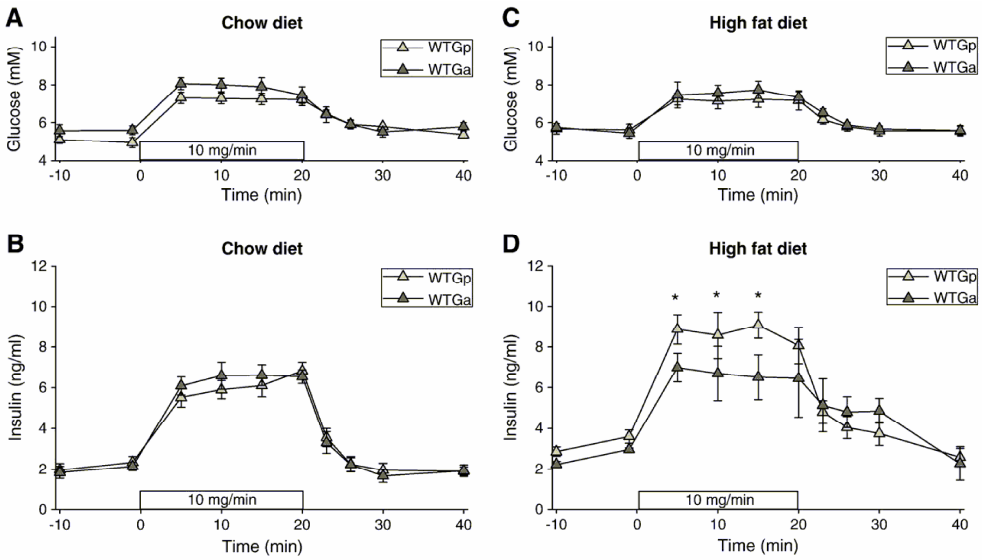

Figure 4: A: Glucose levels before, during and after a 20 minutes glucose infusion in passive and proactive WTG rats fed a chow diet. **B:** Insulin levels before, during, and after a 20 minutes glucose infusion in passive and proactive WTG rats fed a chow diet. **C:** Glucose levels before, during and after a 20 minutes glucose infusion in passive and proactive WTG rats fed a high fat diet. **D:** Insulin levels before, during, and after a 20 minutes glucose infusion in passive and proactive WTG rats fed a high fat diet. Light grey triangles represent passive WTG rats, dark grey triangles represent RHA rats. * indicates a significant difference between WTG passive and proactive WTG rats ($p<0.01$).

3.4. Carcass analysis

The data from the carcass analysis are summarized in table 5. The high fat diet significantly increased all body fat measurements in all groups ($F(7,61)= 7.319$ $p<0.001$). The increased fat content was accompanied by an increase in plasma leptin levels ($F(1,56)= 5.554$ $p<0.05$). There were also differences in fat distribution between passive and proactive rats: passive rats have more central visceral fat, reflected by the higher ratio of epididymal and retroperitoneal fat in the RLAs in comparison to the RHAs ($F(3,33)= 8,203$ $p<0.001$). Finally, we found differences between the Romans (originally bred from a Wistar laboratory rat strain) and the Wild type Groningen rats: the Wild type rats had a significantly lower body fat % and a higher fat free mass ($F(1.53)= 5.278$ $p<0.05$).

Backward regression analysis showed that a model including the coping style, diet, strain, and the epididymal fat mass best predicted the magnitude of the insulin response during the IVGTT ($F(4,73)= 5.234$ $p<0.01$). Further analysis of this interaction showed that the correlation curve of the RLAs has a steeper slope as compared to the curve of the

RHAs. (RHA: R=0.931 B=406.83; RLA: R=0.931 B= 031.23). Passive WTG rats fed the high fat diet display a significant correlation between the percentage epididymal fat and the insulin response. The slope of this correlation curve is similar to the slope of the correlation curve of the proactive roman rats (R=0.598 B=367.43). In the proactive WTG rats no statistical relevant correlation between the insulin response and the amount of epididymal fat was observed.

Table 5: Carcass analysis of RLA, RHA, WTG passive and WTG proactive rats fed standard lab chow or medium fat diet. Fat pads are expressed as grams dry weight. Fat mass and fat free mass are percentage as a percentage of the total carcass weight. Plasma leptin levels were measured in trunk blood. * indicates a significant difference from chow fed rats of the same strain. [a] indicates a significant difference between the Roman and the WTG strain for either passive or pro-active individuals. [B] indicates a significant difference between passive and pro-active individuals within the same strain.

	RLA	RHA	WTG passive	WTG proactive
Epididymal fat (g)				
Chow	4.94 ± 0.32	4.53 ± 0.54	5.20 ± 0.55	5.45 ± 1.26
High fat	6.47 ± 0.62* [B]	5.02 ± 0.26*	6.80 ± 1.06*	6.04 ± 0.62*
Retroperitoneal fat (g)				
Chow	7.77 ± 0.54 [B]	9.71 ± 0.89	7.58 ± 1.33	10.58 ± 1.86
High fat	11.89 ± 0.64*	10.61 ± 1.02*	11.75 ± 1.57*	13.75 ± 2.14*
Subcutaneous fat (g)				
Chow	36.92 ± 1.95	37.66 ± 0.87	31.33 ± 5.33	32.24 ± 2.41
High fat	43.39 ± 1.44*	40.46 ± 1.03*	35.64 ± 3.94*	33.56 ± 2.87
Fat mass (%)				
Chow	13.95± 0.55	15.03 ± 0.54	10.97± 0.51 [a]	10.76 ± 0.42 [a]
High fat	16.48± 0.62*	16.54 ± 0.67*	13.00± 0.51* [a]	12.70± 0.42* [a]
Fat free mass (%)				
Chow	86.05± 2.92	84.97± 3.36	89.03 ± 5.30 [a]	89.24± 5.39 [a]
High fat	83.52 ± 3.83	83.46± 3.66	87.00 ± 5.46 [a]	87.30 ± 5.68 [a]
Plasma leptin (ng/ml)				
Chow	4.46 ± 0.65	3.19 ± 0.76	3.89 ± 0.69	4.05 ± 0.72
High fat	5.46 ± 1.04*	3.64 ± 0.26*	5.42 ± 1.20*	5.62 ± 1.45*

4. Discussion

The present study investigated the interactions between coping style and diet as risk factors for the development of the insulin resistance syndrome. Analogous to findings in humans, we hypothesized that 1) rats with a passive coping strategy are prone to develop insulin resistance, particularly on a diet with an elevated fat content, 2) that rats with a proactive coping style are resistant to develop diet-induced insulin resistance. The results of the present study are largely supportive of these hypotheses.

The first part of the study primarily focused on the influence of personality on the insulin response to an IVGTT. We replicated our previous finding that RLA rats from a strain selectively bred for a passive coping style, were insulin resistant, even on a normal chow diet (9). The present study revealed that providing a high fat diet to RLA rats led to a further increase in the plasma insulin response to an IVGTT. Insulin resistance was less pronounced in passive WTG rats, which are passively coping animals from an unselected rat strain characterized by a high variation in behavior. These rats had normal glucose tolerance and insulin responses on a chow diet, but increased insulin levels on a medium fat diet. Therefore, we conclude that rats characterized by a passive coping style indeed have an increased susceptibility for developing insulin resistance and that this increased susceptibility is primarily associated with differences in personality rather than being the consequence of unspecific genetic selection or drift associated with selective breeding.

The palatable high fat diet led to a significant increase in food intake, body weight, body adiposity and the ratio fat / fat free mass in all groups, irrespective of coping style. To test our hypothesis that rats with a proactive coping style might be resistant to the development of diet-induced insulin resistance we infused a pharmacologically high dose of glucose in both the RLA and RHA rats on both diets. The results were clear. The insulin responses to an IVGTT remained unchanged in the proactive rats, despite a significant increase in food intake and body weight on the high fat diet.

Taken together, we may conclude that passively coping rats are prone to develop insulin resistance, especially in an environment rich in dietary fat and that proactively coping rats are remarkably resistant to diet-induced insulin resistance. The underlying mechanism is, however, still unknown. Differences in the balance of the autonomic nervous system may be an important factor involved. A proactive coping style is strongly associated with high sympathetic and low parasympathetic reactivity while passive coping individuals are characterized by low sympathetic reactivity and high HPA-axis reactivity reflected by high plasma corticosteron responses (16). Since elevated sympathetic outflow directly leads to lowered insulin release (17), one may conclude that the increased sympathetic outflow may

have prevented the elevation of plasma insulin levels in the proactive individuals on the high fat diet. Indeed, we also found, in a separate study, that RHA rats had an increased sympathetic outflow expressed by significantly higher plasma noradrenalin levels than their RLA counterparts (178.1± 25.6 pg/ml in RHA versus 78.7±11.9 pg/ml in RLA ($F(1,13)= 13.146$ $p<0.01$). Similar data were obtained in WTG rats (18), in which elevated noradrenalin outflow was associated with increased sensitivity of presynaptic β2-adrenoceptors in proactive rats.

These differences in autonomic and hormonal outflow between passive and proactive individuals may be secondary to differences in the central nervous system, for example at the level of the serotonergic and dopaminergic modulatory systems in the brain. When compared to passive individuals, proactive rats are characterized by increased serotonergic release and sensitivity (19), and enhanced dopaminergic activity (20). Elevated serotonergic and dopaminergic activity is, in turn,(in)directly associated with increased sympathoadrenal outflow (21-24). Data on the actions and alterations of these systems in proactive versus passive individuals are scarce. It is, however, likely that many other systems (particularly hypothalamic neuropeptides such as NPY and the melanocortins) might be involved as well.

There were remarkable differences in fat storage in rats of the different coping styles. When only considering the weight of epididymal fat pads, it appeared that the passively coping RLA rats, and to a some extent also the WTGp rats, increased their epididymal fat depots when subjected to a high fat diet. This was not the case in the proactive animals. In rodents, epididymal fat deposition has been suggested to be a better predictor for the metabolic syndrome than retroperitoneal, the latter is believed to represent total body fat. Indeed, backward regression analysis in the present study showed that a model including epididymal fat mass, rat strain, diet and coping style best predicted the response to the IVGTT. The insulin response correlated strongly with the epididymal fat content in all rats of the Roman rat strain. The correlation curve of the passive RLAs was skewed towards the left and had a steeper incline as compared to the curve of the proactive RHAs, indicating a strong coping style effect. WTG passive rats fed the high fat diet displayed a similar correlation as observed in the Roman rats strains, whereas in the high fat diet fed WTG proactive rats no significant correlation between the insulin response and the amount of epididymal fat was observed, which suggest that a diet-coping style interaction is important in the WTG rats. Overall, we conclude that the risk factors coping style, diet, and body fat distribution interact to influence glucose homeostasis and insulin sensitivity. An effect that seems independent of body weight or body weight gain.

In conclusion, we observed that passive coping rats were prone to develop insulin resistance, especially in an environment rich in dietary fat. Proactively coping rats were remarkably resistant to diet-induced insulin resistance. Translating this to humans, one might conclude that the passive, type B-like, personality is more susceptible for developing insulin resistance on a palatable fat diet and that the proactive, type A-like, personality seems to be protected against the development of diet-induced insulin resistance. Indeed, data from literature confirm that several of the physiological and behavioral characteristics associated with a passive personality, like increased HPA-axis activity (25;26) and increased anxiety traits (27;28), are risk factors for the development of insulin resistance. Unfortunately, evidence in literature for an interaction between personality and the risk to develop insulin resistance is less clear. In fact, the studies on risk factors for type 2 Diabetes using questionnaires to assess personality are conflicting (5;29-31), but one may argue that particularly questionnaire-based studies are less suited for a physiological characterization of patients at risk. However, this paper may be viewed as a useful starting point to investigate interactions between personality and metabolic diseases in humans.

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Reference list

1. Kelley DE. Overview: What Is Insulin Resistance? *Nutr Rev* 2000; 58(3 Pt 2): S2-S3.

2. Woods SC, Seeley RJ, Rushing PA, D'Alessio D, Tso P. A Controlled High-Fat Diet Induces an Obese Syndrome in Rats. *J Nutr* 2003; 133(4): 1081-7.

3. Shiraev T, Chen H, Morris MJ. Differential Effects of Restricted Versus Unlimited High-Fat Feeding in Rats on Fat Mass, Plasma Hormones and Brain Appetite Regulators. *J Neuroendocrinol* 2009; 21(7): 602-9.

4. Colman E, Toth MJ, Katzel LI, Fonong T, Gardner AW, Poehlman ET. Body Fatness and Waist Circumference Are Independent Predictors of the Age-Associated Increase in Fasting Insulin Levels in Healthy Men and Women. *Int J Obes Relat Metab Disord* 1995; 19(11): 798-803.

5. Sovio U, King V, Miettunen J, Ek E, Laitinen J, Joukamaa M, Veijola J, Jarvelin MR. Cloninger's Temperament Dimensions, Socio-Economic and Lifestyle Factors and Metabolic Syndrome Markers at Age 31 Years in the Northern Finland Birth Cohort 1966. *J Health Psychol* 2007; 12(2): 371-82.

6. Yancura LA, Aldwin CM, Levenson MR, Spiro A, III. Coping, Affect, and the Metabolic Syndrome in Older Men: How Does Coping Get Under the Skin? *J Gerontol B Psychol Sci Soc Sci* 2006; 61(5): 295-303.

7. Feldman PJ, Steptoe A. Psychosocial and Socioeconomic Factors Associated With Glycated Hemoglobin in Nondiabetic Middle-Aged Men and Women. *Health Psychol* 2003; 22(4): 398-405.

8. Raikkonen K, Hautanen A, Keltikangas-Jarvinen L. Association of Stress and Depression With Regional Fat Distribution in Healthy Middle-Aged Men. *J Behav Med* 1994; 17(6): 605-16.

9. Boersma GJ, Scheurink AJ, Wielinga PY, Steimer TJ, Benthem L. The Passive Coping Roman Low Avoidance Rat, a Non-Obese Rat Model for Insulin Resistance. *Physiol Behav* 2009; 97(3-4): 353-8.

10. Bignami G. Selection for High Rates and Low Rates of Avoidance Conditioning in the Rat. *Anim Behav* 1965; 13(2): 221-7.

11. Steffens AB. A method for frequent sampling of blood and continuous infusion of fluids in the rat without disturbing the animal. 4, 833-836. 1969. physiology and behavior.

12. Steffens AB. Blood Glucose Levels and Food Intake in Normal and Hypothalamic Hyperphagic Rats. *Acta Physiol Pharmacol Neerl* 1967; 14(4): 524-6.

13. Pinel JPJ, Treit D. Burying as a defensive response in rats *J. Comp. Physiol. Psychol.* 2010. 92, 708-712..

14. Strubbe JH, Bouman PR. Plasma Insulin Patterns in the Unanesthetized Rat During Intracardial Infusion and Spontaneous Ingestion of Graded Loads of Glucose. *Metabolism* 1978; 27(3): 341-51.

15. Hoffman WS. A rapid photoelectric method for the determination of glucose in blood and urine. 120, 51-55. 1937. Journal of biological chemistry.

16. Koolhaas JM, Korte SM, de Boer SF, van d, V, Van Reenen CG, Hopster H, De J, I, Ruis MA, Blokhuis HJ. Coping Styles in Animals: Current Status in Behavior and Stress-Physiology. *Neurosci Biobehav Rev* 1999; 23(7): 925-35.

17. Rohner-Jeanrenaud F, Bobbioni E, Ionescu E, Sauter JF, Jeanrenaud B. Central Nervous System Regulation of Insulin Secretion. *Adv Metab Disord* 1983; 10: 193-220.

18. Smit J, van Oosten RV, Palm IF, Doze P, Koolhaas JM, zaagsma J. Prejunctional modulation of noradrenergic neurotransmission in wild type rats: a possible relationship with coping style. PhD-thesis, 1997; chapter 7: 111-122

19. Giorgi O, Piras G, Lecca D, Hansson S, Driscoll P, Corda MG. Differential Neurochemical Properties of Central Serotonergic Transmission in Roman High- and Low-Avoidance Rats. *J Neurochem* 2003; 86(2): 422-31.

20. Driscoll P, Dedek J, Martin JR, Zivkovic B. Two-Way Avoidance and Acute Shock Stress Induced Alterations of Regional Noradrenergic, Dopaminergic and Serotonergic Activity in Roman High- and Low-Avoidance Rats. *Life Sci* 1983; 33(17): 1719-25.

21. Dronjak S, Spasojevic N, Gavrilovic L, Varagic V. Effects of Noradrenaline and Serotonin Reuptake Inhibitors on Pituitary-Adrenocortical and Sympatho-Adrenomedullar System of Adult Rats. *Neuro Endocrinol Lett* 2007; 28(5): 614-20.

22. Jensen I, Llewellyn-Smith IJ, Pilowsky P, Minson JB, Chalmers J. Serotonin Inputs to Rabbit Sympathetic Preganglionic Neurons Projecting to the Superior Cervical Ganglion or Adrenal Medulla. *J Comp Neurol* 1995; 353(3): 427-38.

23. Nagahama S, Ann HS, Chen YF, Lindheimer MD, Oparil S. Role of Vasopressin in the Cardiovascular Effects of LY171555, a Selective Dopamine D2 Receptor Agonist: Studies in Conscious Brattleboro and Long-Evans Rats. *J Pharmacol Exp Ther* 1987; 242(1): 143-51.

24. Spasojevic N, Gavrilovic L, Kovacevic I, Dronjak S. Effects of Antidepressants Maprotiline and Fluxilan on Sympatho-Adrenomedullary System in Stressed Rats. *Auton Neurosci* 2009; 145(1-2): 104-7.

25. Asensio C, Muzzin P, Rohner-Jeanrenaud F. Role of Glucocorticoids in the Physiopathology of Excessive Fat Deposition and Insulin Resistance. *Int J Obes Relat Metab Disord* 2004; 28 Suppl 4: S45-S52.

26. Qi D, Rodrigues B. Glucocorticoids Produce Whole Body Insulin Resistance With Changes in Cardiac Metabolism. *Am J Physiol Endocrinol Metab* 2007; 292(3): E654-E667.

27. Carroll JF, Franks SF, Smith AB, Phelps DR. Visceral Adipose Tissue Loss and Insulin Resistance 6 Months After Laparoscopic Gastric Banding Surgery: a Preliminary Study. *Obes Surg* 2009; 19(1): 47-55.

28. Narita K, Murata T, Hamada T, Kosaka H, Sudo S, Mizukami K, Yoshida H, Wada Y. Associations Between Trait Anxiety, Insulin Resistance, and Atherosclerosis in the Elderly: a Pilot Cross-Sectional Study. *Psychoneuroendocrinology* 2008; 33(3): 305-12.

29. Niaura R, Banks SM, Ward KD, Stoney CM, Spiro A, III, Aldwin CM, Landsberg L, Weiss ST. Hostility and the Metabolic Syndrome in Older Males: the Normative Aging Study. *Psychosom Med* 2000; 62(1): 7-16.

30. Raikkonen K, Hautanen A, Keltikangas-Jarvinen L. Association of Stress and Depression With Regional Fat Distribution in Healthy Middle-Aged Men. *J Behav Med* 1994; 17(6): 605-16.

31. Ravaja N, Keltikangas-Jarvinen L, Viikari J. Type A Behavior and Metabolic Syndrome Precursors in Young Adults. *J Clin Epidemiol* 1996; 49(3): 335-43.