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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 proactive personality might be protected against the development of diet-induced insulin resistance.

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.

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2. 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.

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 (24×24×36 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 ($T=20$ °C, 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].

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	10 mg/ml and 15 mg/ml
RHA	8	Chow	10 mg/ml and 15 mg/ml
WTG p	8	Chow	10 mg/ml
WTG a	8	Chow	10 mg/ml
WTG p	8	High fat	10 mg/ml
WTG p	8	High fat	10 mg/ml
RLA	8	High fat	15 mg/ml
RHA	8	High fat	15 mg/ml

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 24×24×36 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 min intravenous infusion of 10 mg glucose in 0.1 ml saline per minute (total 200 mg glucose in 2 ml saline) or a 30 min 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 min. After the start of the infusion at $t=0$ min, blood samples were taken at time points $t=5$, 10, 15, 20, 25, 30, and 40 min. During IVGTT2 the rats were infused with 15 mg/min glucose over a 30 min period. Again, two baseline samples were taken at time points $t=-11$ and -1 min. 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 min.

Blood samples were kept on ice and stored in files with EDTA (0.09 g/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 min 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