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Original article

Reversibility of hyperglycaemia and islet abnormalities in the high fat-fed female ZDF rat model of type 2 diabetes

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ABSTRACT

Introduction: We characterised the development of Type 2 diabetes and associated changes in islet appearance in female ZDF rats and explored its suitability for studies with novel therapeutic agents. **Methods:** Female ZDF rats were either chow or high fat (60%) fed for up to 36 days and blood glucose and plasma insulin concentration measured. Additionally, we restored two groups of rats back to chow diet after ten and nineteen days of high fat feeding to determine the reversibility. Finally, two other groups of high fat-fed animals were dosed either orally with drug vehicle or had a minipump implanted subcutaneously to determine the effect of dosing method upon the progression of this disease model. The beta cell mass and morphology were assessed by immunohistochemistry for insulin. **Results:** High fat feeding elevated blood glucose compared to chow-fed controls which peaked by 15 days, and maintained throughout the study. Plasma insulin reached a maximum after 8 days, but declined over the remaining 4 weeks. Assessment of islets revealed marked disruption, dispersion and weaker insulin staining. The area and percentage β-cells were higher in high fat-fed animals. High fat diet treatment reversal when animals were moderately hyperglycaemic, when plasma insulin was still elevated, reversed the hyperglycaemia and maintained islet morphology similar to that of chow-fed animals. In contrast, dietary reversal when plasma insulin was declining, did not prevent continual decline in plasma insulin, β-cell mass or islet disruption. Oral dosing tended to increase blood glucose and decrease plasma insulin whereas administration by minipump lowered blood glucose. **Discussion:** The obese female ZDF rat offers the opportunity for preclinical evaluation of novel therapies directed towards improving pancreatic function, provided treatment is initiated prior to the precipitous decline in insulin production. Caution should be exercised in comparison of compounds administered by different dosing routes however.

1. Introduction

Type 2 diabetes results from progressive failure of pancreatic insulin secretion to compensate for increased peripheral insulin resistance and maintain normoglycaemia. Data from the United Kingdom Prospective Diabetes (UKPDS) showed that reduced pancreatic function is apparent several years before the onset of hyperglycaemia (Holman, 1998; Matthews, Cull, Stratton, Holman, & Turner, 1998). Either inadequate insulin reserve or impaired β-cell responsiveness to glucose or other secretagogues may contribute to pancreatic dysfunction; the former could result from reduced β-cell mass or a defect in insulin synthesis (Kahn, 2001). Techniques for monitoring β-cell mass in human subjects are not yet available, however autopsy of a small number of type 2 diabetics revealed that β-cell mass was reduced (Butler, Janson, Bonner-Weir, Ritzel, Rizza, & Butler, 2003; Deng, Vatamaniuk, Huang, Doliba, Lian, Frank et al., 2004). In contrast, in obese, insulin-resistant nondiabetic subjects β-cell mass was increased (Butler et al., 2003). Elevated circulating glucose or lipid concentrations may themselves contribute to changes in β-cell mass and responsiveness (Paris, Bernard-Kargar, Berthault, Bouwens, & Ktorza, 2003; Poitout & Robertson, 2002).

Novel drug therapies to address declining pancreatic function are now being sought, and a well-characterised animal model in which these processes can be quantified is an essential tool in their development.

Several preclinical models have been used to explore the effects of novel compounds on pancreas failure, most frequently the male Zucker Diabetic Fatty (ZDF) rat (for example Bergeron, Yao, Woods, Zycband, Liu, Li et al., 2006; Smith, Lister, Toseland, & Buckingham, 2000; Sturis, Gotfredsen, Romer, Rolin, Ribel, Brand et al., 2003; Zhou,

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Madjidi, Wilson, Nothhelfer, Johnson, Palma et al., 2005) and the db/db mouse (Han, Chung, Cheon, Rhee, Yoon, Lee et al., 2009; Kawasaki, Matsuda, Kanda, Inoue, & Kaku, 2005; Kjorholt, Akerfeldt, Biden, & Laybutt, 2005; Tozzo, Ponticiello, Swartz, Farrelly, Zebo, Welzel et al., 2007) however both these models have limitations as models of pancreatic degeneration in man. Type 2 diabetes in man is invariably associated with a high fat, high calorie diet and arises progressively over a considerable period of time (UK Prospective Diabetes Study (UKPDS) Group, 1998). Male ZDF rats develop hyperglycaemia spontaneously on normal rodent chow diets at about 6–7 weeks of age, following β-cell apoptosis which leads to loss of β-cell mass (Etgen & Oldham, 2000; Pick, Clark, Kubstrup, Levisetti, Pugh, Bonner-Weir et al., 1998). Practically, this presents challenges to the investigator: by preserving insulin sensitivity, intervention before this stage has been shown to preserve islet architecture (Finegood, McArthur, Kojwang, Thomas, Topp, Leonard et al., 2001) whereas later intervention has no effect (Wargent, Stocker, Augstein, Heinke, Meyer, Hoffmann et al., 2005). Similar challenges are posed when working with db/db mice. Goto-Kakisaki (GK) rats have occasionally been employed but variable results observed between colonies (reviewed in Portha, Lacraz, Kergoat, Homo-Delarche, Giroix, Bailbe et al., 2009). Models where pancreas- or β-cell mass are reduced chemically with streptozotocin or surgery have been utilised to explore specific anti-apoptotic or proliferative mechanisms (Mu, Woods, Zhou, Roy, Li, Zycband et al., 2006); these models show dissociation between β-cell mass and functionality (Kargar & Ktorza, 2008). In contrast to male ZDF rats, female ZDF rats only become severely hyperglycaemic when fed a high fat (48% kcal) diet, as a result of markedly reduced plasma insulin concentration (Corsetti, Sparks, Peterson, Smith and Sparks, 2000). A moderate (29.5%) fat content, maintained insulin hyper-secretion, and resulted in only moderate hyperglycaemia. In a more detailed study, insulin production decreased rapidly after 1–2 weeks of the high fat diet (Zhou et al., 2005). Furthermore, not only was insulin content of pancreatic islets reduced, but islet remodelling was observed, with loss of normal morphology and the appearance of dense, collagen fibres within the islet core.

Investigation of novel pancreas-directed therapies *in vivo* requires reliable and reproducible methods of quantification of both β-cell morphology and function. Histological methods and a mathematical (βIG) model to estimate insulin sensitivity and β-cell function from *in vivo* studies have been described (Topp, Atkinson & Finegood, 2007); we sought to complement this by quantifying the visible changes in islet morphology such as those described by Zhou et al. (2005).

Based on the observation that susceptibility to hyperglycaemia depended on dietary fat content, we hypothesised that, in the female ZDF rat, reversal of the high fat challenge would mimic an effective therapy. The objective of this study was to characterise glycaemic control and β-cell morphology after high fat challenge, and to explore the plasticity of the pancreatic failure to dietary reversal. This is the first time the β-cell changes have been evaluated in this particular model of progressive pancreatic adaptation and degeneration. Thus we would establish the suitability of the high fat-fed female ZDF rat for characterisation of novel therapies directed at β-cell dysfunction.

2. Research design and methods

2.1. Animals

Female obese ZDF rats (Gmi-*fa*/*fa*) rats and lean ZDF (Gmi-*fa*/+) were obtained from Charles River Laboratories, Belgium at 6–7 weeks of age. Animals were housed two per cage with free access to food and water on a 12 h:12 h light:dark cycle (lights off 11:00 am) throughout the whole study. They were allowed to acclimatise on standard rodent chow (RM1; Special Diet Services UK Ltd.) for 3 weeks before the start of the experiment. Lean rats were maintained on chow diet throughout the study. On day 1 of the study the obese rats were assigned to either chow ($n=8$) or high fat diet (C13004; 48% kcal as fat, Research Diets, Inc., New Jersey, USA) ($n=48$). Based upon glycated haemoglobin (GHb), blood glucose, body weight and food intake data obtained during acclimatization animals were assigned to specific treatment groups. This was to ensure that the animal groups started with the same mean values on day 1 of the study. C13004 diet used in this study has subsequently been redesignated C13004WG (Research Diets, personal communication). Details of treatment regimes are given in Fig. 1.

All work was carried out in accordance with the U.K. Home Office Animals (Scientific Procedures) Act 1986.

2.2. Monitoring

Disease progression was monitored in conscious animals by measurement of glycated haemoglobin, blood glucose and plasma

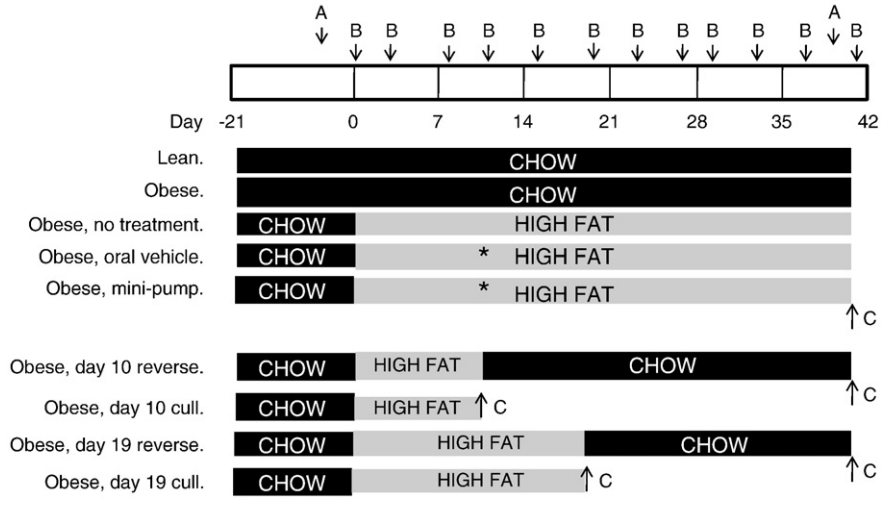

Fig. 1. Description of the treatment for the different groups of ZDF rats. Female ZDF rats were 6–7 weeks of age at the start of the study. Following a 3 week acclimatisation period the animals were assigned to the following groups: Lean, chow-fed ($n=8$); Obese, chow-fed ($n=8$); Obese high fat-fed ($n=10$); Obese high fat-fed, oral vehicle treated ($n=10$); Obese high fat-fed, min-pump implanted ($n=8$); Obese high fat-fed, reversed day 10 ($n=4$); Obese high fat-fed, culled day 10 ($n=4$); Obese high fat-fed, reversed day 19 ($n=4$); Obese high fat-fed, culled day 19 ($n=4$). Animals were assigned to groups based upon glycated haemoglobin, blood glucose, bodyweight and food intake measurements obtained during the baseline period. Gycated Hb (A), blood glucose and plasma insulin (B), start of oral dosing or minipump implant (*) and sacrifice of animals (C) is indicated.