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Increased lipids in non-lipogenic tissues are indicators of the severity of type 2 diabetes in mice

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Abstract

We hypothesised that the molecular changes triggered in type 2 diabetes might cause phenotypic changes in the lipid fraction of tissues. We compared tissue lipid profiles of inbred lean B6-Bom with those of the obese B6-*ob*/*ob* and diabetic BKS-*db*/*db* mice and found that genetically diabetic mice significantly accumulate fat (especially monounsaturated fatty acids, MUFA) in non-lipogenic tissues such as the eye (MUFA, 2-fold), skeletal muscle (MUFA, 13-fold) and pancreas (MUFA, 16-fold). In contrast, the B6-*ob*/*ob* mice which manifest a milder form of type 2 diabetes use the liver as their predominant lipid depot (MUFA 91-fold increase, as compared to lean mice values). The lipids in the BKS-*db*/*db* skeletal muscle and pancreas were also significantly enriched with linoleic acid (LA, (9-fold and 6-fold, respectively); and alpha-linolenic acid (ALA, 8.5-fold and 8-fold, respectively). MUFA, LA and ALA accumulation in the non-lipogenic tissues of BKS-*db*/*db* mice was associated with reduced liver stearoyl-CoA desaturase-1 expression.

1. Introduction

Lipids constitute a major part of mammals where they provide the physical barrier that compartmentalises the single cells, as well as serve as a major storage form of energy in the liver and adipose tissue. Membrane lipids do not just form an inert framework for cells. Rather, fundamental roles are now recognised for several lipids and their bioactive derivatives in cell function, especially in the responses of the cell to external stimuli from hormones, neurotransmitters and growth factors. It is therefore not surprising that alterations in lipid composition have been associated with disease conditions such as diabetes and obesity [1,2].

Obesity is now known as the major risk factor for the development of type 2 diabetes. However, not all cases of obesity lead to the development of full blown type 2 diabetes. Gene expression studies aimed at distinguishing between two types of obesity—that which resists the onset of type 2 diabetes, and that which leads to the overt disease, suggest a role for hepatic lipogenic capacity [3]. Thus a resistance to type 2 diabetes has been found to correlate with a high and sustained level of hepatic lipogenesis in non-insulin dependent diabetes mellitus (NIDDM) model mice [4].

The identification of patients at risk for the development of type 2 diabetes is essential for early detection, prevention and treatment. In this study, we sought to define the severity of type 2 diabetes in two NIDDM mice models in terms of the fatty acid profiles of tissues. Most studies have relied on data from the analyses of

Abbreviations: NIDDM, Non-insulin-dependent diabetes mellitus; LA, Linoleic acid; ALA, Alpha-linolenic acid; SFA, Saturated fatty acid; MUFA, Monounsaturated fatty acid; Liver-TAG, Liver-triglyceride fraction; Liver-PL, Liver-phospholipid fraction; AA, Arachidonic acid; DHA, Docosahexaenoic acid

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serum or blood constituents as samples can be obtained in a relatively less-intrusive manner. From our studies of the anti-diabetic properties of the herbal remedy made from *Rauvolfia vomitoria* and *Citrus aurantium* [5], we found that the reduction in tissue lipid content following treatment of genetic diabetic mice with the plant extract was accompanied by an increase in serum triglyceride content. Thus we hypothesised that while serum constituents may present a snapshot of overall dynamics of the levels of metabolites, the tissues are more likely to represent the end-point of the interaction of the organism and its environment. While such studies cannot be carried out in man, investigation of the development of these processes in mice might lead to the identification of new targets for early diagnosis and therapy of type 2 diabetes.

Hence, we investigated the fatty acid (FA) content of some of the tissues implicated in the pathophysiology of type 2 diabetes in mice. We compared the fatty acid composition of some tissues associated with the complications of diabetes in the genetically bred diabetic C57BLKS-*db*/*db* (BKS-*db*/*db*) and obese C57BL/6J-*ob*/*ob* (B6-*ob*/*ob*) mice with those from lean C57BL/6J (B6-Bom) mice of the same age and fed on the same diet. The leptin-deficient B6-*ob*/*ob* mice are phenotypically similar to their lean littermates (B6-*ob*/+ and B6-+/+) at birth but begin to differ around the age of 26 days. The obese mice are characterised by marked obesity, hyperphagia, reduced thermogenesis, transient hyperglycemia and elevated plasma insulin concentrations due to an increase in number and size of the beta cells in the pancreas [6,7]. The BKS-*db*/*db* mice produce leptin but lack the receptor for the hormone in the hypothalamus. Thus, the genetic diabetic mice are also hyperphagic and rapidly increase body weight from age 4 weeks until they are 10 weeks old. This fast-growth period coincides with an early hyperinsulinemic phase, which is followed by a hypoinsulinemic phase at age 2–3 months, when the pancreas begins to degenerate. In addition, the BKS-*db*/*db* mice have abnormalities in the immune system and develop a more severe form of NIDDM with clinical features that include massive necrosis of pancreatic beta cells, impairment of kidney function and myocardial disease [8–10]. BKS-*db*/*db* mice have therefore been used as model animals for studying processes like atherosclerosis [11], impaired wound healing [12] and high blood pressure [13] that are hallmarks of the metabolic syndrome. The tissues investigated for their FA profiles include adipose tissue, eye, liver, pancreas and skeletal muscle. Our results show that while there was an overwhelming deposition of triglycerides in the non-lipogenic tissues like skeletal muscle, eye and pancreas in 18–20 week old BKS-*db*/*db* mice, B6-*ob*/*ob* mice of the same age had primarily, a very fatty liver. Interestingly, the liver was not used as a major lipid sink in the BKS-*db*/*db* mice.

2. Materials and methods

2.1. Experimental animals

The study was performed under the guidelines approved by the Danish Animal Care and Use license. The experimental animals were male inbred B6-*ob*/*ob* ($n = 9$), BKS-*db*/*db* ($n = 8$) and B6-Bom mice ($n = 9$). The animals were purchased from Taconic M & B A/S, Ry Denmark, when they were 13 weeks old and kept in groups of 3 mice per 25 cm × 40 cm cages with wire-mesh tops. The animals were housed in a room kept under controlled environmental conditions (temperature 20 ± 2 °C, relative humidity 55 ± 5%, with a light/dark cycle of 12 h dark each and air changes of 10 times per hour), and allowed free access to water and food. All the animals were fed the Altromin C1314 standard mice/rat diet containing 23.5% protein, 5% fat, 4% fibre, 6.4% ash, 11% moisture, 6% disaccharide, 34% polysaccharide and 2825 kcal/kg metabolisable energy. The fatty acid composition of the diet (mg kg^{-1}) was palmitic acid, 5944; palmitoleic acid, 126, stearic acid, 1706, oleic acid, 10221, linoleic acid, 23294, alpha-linolenic acid, 3197, arachidic acid, 200, eicosanoic acid, 406, arachidonic acid, 546, eicosapentanoic acid, 243 (Altromin, Lage, Germany).

2.2. Sample collection and biochemical analysis

When the animals were 18–20 weeks old, they were weighed, ether anaesthetised and blood samples were collected by orbital puncture before the animals were sacrificed. The blood samples were allowed to clot at room temperature (about an hour) and cleared serum samples were collected after centrifugation at 3000*g*, 4 °C for 30 min. Serum samples were then stored at −20 °C until analysed. Serum glucose, triglyceride and cholesterol contents were determined on the Boehringer Mannheim/Hitachi analytical system. Serum insulin and glucagon contents were determined using RIA Kits, (Linco Research, Inc. Missouri, USA). Samples of the whole eye with optic nerve, liver (left anterior lobe), pancreas, gastrocnemius muscle, and adipose tissue (from around the testis) were taken from each of the animals, frozen in liquid nitrogen and stored at −70 °C until further analysed.

2.3. Fatty acid analysis

Tissue samples were homogenised and total lipids together with an internal standard were extracted with chloroform/methanol, methylated and analysed by gas-liquid chromatography (GLC) in a Hewlett-Packard 6890 series Chromatograph with flame-ionisation detection (Hewlett-Packard GmbH, Waldbronn, Germany), essentially as earlier described [14]. The total lipids from