Features of the Metabolic Syndrome in the Spontaneously Hypertriglyceridemic Wistar Ottawa Karlsburg W (RT1^u haplotype) Rat

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The aim of this study was to characterize the Wistar Ottawa Karlsburg W ([WOKW] RT1^u haplotype) rat in a cross-sectional study (up to 14 weeks of age) for traits with pathophysiological relevance to the metabolic syndrome in comparison to the Dark Agouti (DA) rat, to determine the age at which the WOKW rat begins to manifest the characteristics of the metabolic syndrome. The findings indicate that the WOKW rat is dyslipidemic (high serum triglycerides and low high-density lipoprotein [HDL] cholesterol), hyperinsulinemic, and obese. The interval between 8 and 10 weeks appears to be the crucial age after which the most dramatic changes were observed in the measured phenotypic traits in the WOKW rat, as well as the most expressive differences between the WOKW and DA strains. Considering the phenotypic differences between WOKW and DA rats, the DA rat provides an appropriate control strain for crossing studies with the WOKW rat, which might contribute to the explanation of the genetic basis for traits of the metabolic syndrome in this model.

SPONTANEOUSLY DIABETIC BioBreeding (BB) rats, a well-known animal model of type 1 diabetes mellitus, and animals from their founder outbred Wistar rat stock were transferred from the BioBreeding Laboratories in Ottawa, Ontario, Canada, to the animal facility at the Institute of Pathophysiology (formerly the Institute of Diabetes Gerhardt Katsch) in Karlsburg, Germany in 1981.[1] Genetic studies with BB rats showed that they were homozygous for the RT1^u haplotype of the major histocompatibility complex, while the animals from outbred stock carried both haplotypes RT1^a and RT1^u. Subsequent crossing studies indicated that the RT1^u haplotype is one of the predisposing factors for the development of diabetes in BB rats.[2,3]

To investigate the impact of the RT1^u haplotype in rats without the complete genetic background of the BB rat, we selected RT1^a and RT1^u homozygous animals of the outbred Wistar rat stock and generated 2 inbred lines carrying either the diabetes-susceptible RT1^u or diabetes-resistant RT1^a haplotype. The lines were designated Wistar Ottawa Karlsburg A ([WOKW] RT1^a) and WOKW (RT1^u) and were inbred separately. After more than 35 generations of inbreeding, a decrease of fertility and pup survival in WOKW rats was observed, which led to the first characterization of WOKW rats for metabolic traits and blood pressure compared with nondiabetic BB/OK rats, both descending from the same founder Wistar rat stock. In comparison to nondiabetic BB/OK rats, WOKW rats developed impaired glucose tolerance and were hyperinsulinemic, hypertriglyceridemic, and moderately hypertensive but did not develop diabetes,[4] since they do not carry the same genetic background as the BB/OK rat. The WOKW rat was therefore postulated to be a useful animal model for the genetic and physiologic analysis of the metabolic syndrome, which is characterized by insulin resistance, hyperinsulinemia, obesity, impaired glucose tolerance, dyslipidemia, and hypertension. With respect to the lack of animal models with polygenic determined features of the metabolic syndrome, a genetic analysis of the syndrome using crossing studies with the WOKW rat could contribute to the explanation of the genetic basis for this complex disorder. To be most successful in a crossing study, the parental strains should strongly differ by phenotype. As mentioned before, in the first comparative study with the WOKW rat, the BB/OK rat was used.[4] Regarding the same origin as the WOKW rat, the BB/OK rat would not provide an appropriate strain for crossing studies with the WOKW rat. However, previously observed phenotypic differences between Dark Agouti (DA) and WOKW rats for body weight in our animal facilities prompted us to investigate whether the DA rat could be a suitable phenotypic control for the WOKW rat for further traits of the metabolic syndrome.

Since in the first study, the WOKW rat was characterized at 18, 22, and 26 weeks, the question arises as to the age at which the WOKW rat begins to manifest the signs of the metabolic syndrome. Therefore, in this investigation, a cross-sectional comparative study was undertaken between WOKW and DA rats from week 4 to week 14 of life.

MATERIALS AND METHODS

Animals, Housing, and Feeding

Twelve male and 12 female WOKW/K (designated WOKW; Karlsburg, Germany) and DA/K (designated DA; Karlsburg, Germany) rats at age 4, 6, 8, 10, 12, and 14 weeks were characterized in a cross-sectional study for traits with pathophysiological relevance to the metabolic syndrome. Males and females were kept separately in groups of 3 in Macrolon (Ehret, Einmendingen, Germany) cages under strict hygienic conditions and were free of major pathogens as described previously.[5] They had free access to food (Ssniff R; Soest, Germany) and acidulated water and were maintained on a 12-hour light/dark cycle (5 AM/5 PM).

Phenotypic Characterization

The body weight and length of the rats were determined to calculate the body mass index (BMI). Blood samples were obtained from fed WOKW and DA conscious rats by puncturing the ophthalmic venous plexus between 7 and 9 AM. Serum triglycerides, high-density lipoprotein (HDL) cholesterol, and total cholesterol were analyzed using an automatic analyzer (Roche Cobas Mira Plus, Roche, Basel, Switzer-

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land). Serum leptin and insulin were determined using radioimmunoassay (RIA) kits (Rat Insulin RIA Kit and Rat Leptin RIA Kit; Linco Research, St. Charles, MO). Rats were killed by pentobarbital injection, and the left and right inguinal adipose pads were removed and weighed. The sum of the adipose pads relative to the body weight and multiplied by 100 comprised the adiposity index.

All experiments were performed in accordance with the Rules for Animal Care of the Ministry of Nutrition, Agriculture, and Forestry of the Bundesministerium für Ernährung, Landwirtschaft und Forsten and were approved by the Institution's animal care and use committee.

Data Analysis

The values are reported as the mean ± SD. Differences for each trait between WOKW and DA rats were assessed by ANOVA using the SPSS computer program (SPSS, Chicago, IL).

RESULTS

WOKW rats were significantly heavier than DA rats. However, the body weight of WOKW rats increased rapidly at an age interval of 6 to 10 weeks in both sexes (Fig 1A). A similar tendency was also observed for the BMI (Fig 1B), as well as the adiposity index (Fig 2A), showing significantly higher values in WOKW versus DA rats from 10 weeks of age for males (from 8 weeks for the adiposity index) and from 8 weeks for females (Fig 1B). From 10 weeks of age, WOKW males had higher serum leptin levels than DA rats, but there were no differences between females of either strains except for the 8th week of age ($P < .05$; Fig 2B).

Serum insulin was significantly higher in WOKW versus DA rats from 10 weeks in males, while only 10-week-old females showed significant differences between both strains (Fig 3A). Serum triglyceride levels were significantly higher in the WOKW rat at all age intervals, with an apparent increase observed after 4 weeks of age for both sexes (Fig 3B). Furthermore, regarding serum cholesterol and HDL cholesterol, the WOKW rat showed lower levels than the DA rat for both traits in both sexes, although a decrease of values with age was evident (Fig 4A and B).

With respect to sex differences, obviously more were found for the WOKW rat versus the DA rat. In the WOKW rat, significant sex differences were found for the body weight, BMI, adiposity index, serum leptin, serum insulin, triglycerides, and total cholesterol, but in the DA rat, only the body weight, BMI, serum total cholesterol, and HDL cholesterol showed significant sex differences. WOKW males showed significantly higher values than WOKW females in all traits. For DA rats, males had a significantly higher BMI but lower serum total cholesterol and HDL cholesterol than females.

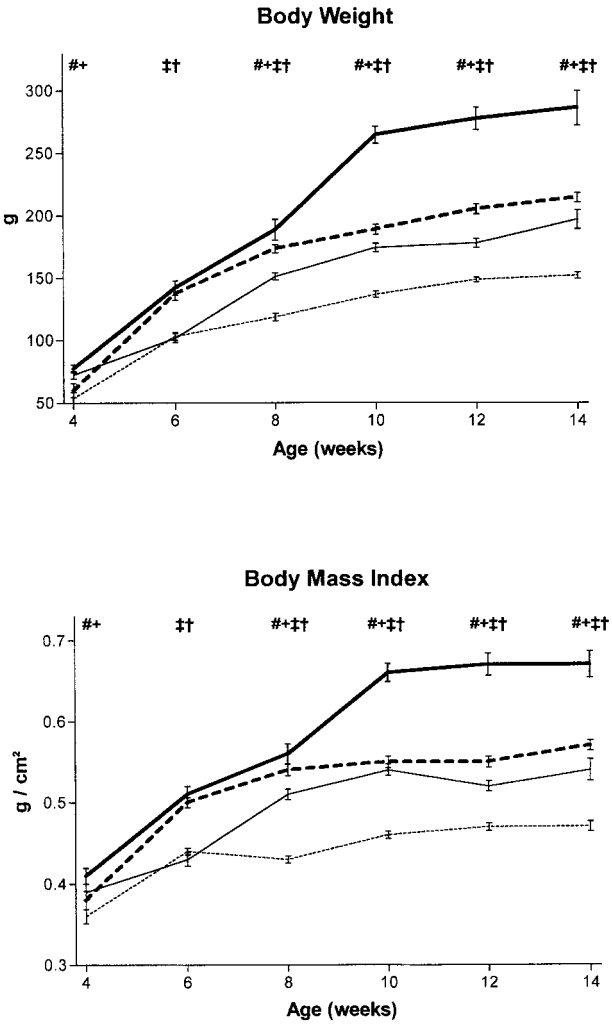

Fig 1. (A) Body weight and (B) body mass index in WOKW and DA rats (males and females). #WOKW *v* DA males ($P < .05$), ⁺WOKW *v* DA females ($P < .05$), ‡WOKW males *v* females ($P < .05$), †DA males *v* females ($P < .05$). ▬ ▬ ▬, DA male; ·····, DA female; ▬▬, WOKW male; ———, WOKW female.

DISCUSSION

In 1985, Modan et al[6] epidemiologically established that hyperinsulinemia constitutes a link between glucose intolerance, obesity, hypertension, and dyslipidemia. Reaven[7] used the term ''syndrome X'' and Kaplan[8] the term ''deadly quartet'' for this condition, which is the main cause of ill health in industrial societies. With respect to the complexity of the syndrome and its genetic heterogeneity in the human, experimental models can enable the study of the pathophysiological mechanisms and their genetic basis. As stated by Shafrir,[9] animals with characteristics of the plurimetabolic syndrome can be divided into 2 groups: nutritionally induced and genetically determined animal models. Regarding the first group, it was supposed that animals kept on a high-energy diet and developing features of the metabolic syndrome could be appropriate models, since the syndrome is typical for populations with dietary abundance. The disadvantage of these models is that a certain diet, eg, sucrose intake, can produce different effects in various strains, indicating the diversity of the metabolic syndrome, as well as its dependence on the genetics of these strains.[9] On the other hand, there are genetically determined models of insulin resistance controlled by single gene mutations (*obese*, *fat*, and *corpulent*),[10] while the features of the syndrome in humans seem to be under polygenic control. Therefore, a search for new animal models manifesting features of the metabolic syndrome and its genetic and physiologic characterization is a great challenge. We currently report a phenotypic characterization of the WOKW rat, a model of the metabolic syndrome, compared with the DA rat in a cross-sectional study from week 4 to week 14 of age. In

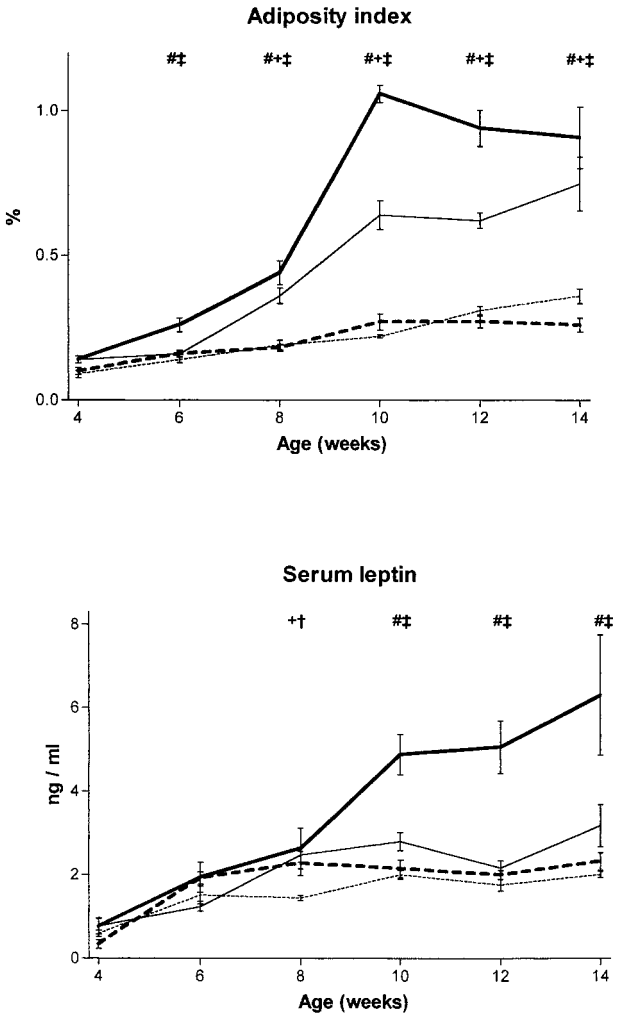

Fig 2. (A) Adiposity index and (B) serum leptin in WOKW and DA rats (males and females). See Fig 1.

comparison to the previous study reporting the WOKW rat as a potential animal model of the metabolic syndrome, this study shows additional characteristics of the WOKW rat, as well as their age-dependent profiles.

The body weight and BMI were significantly higher in the WOKW rat versus the DA rat, implying that the WOKW rat is obese. This is in contrast to the first study in which no obesity was postulated in the WOKW rat, based on a simple comparison of body weight between WOKW and BB/OK rats.[4] Therefore, in this study, the BMI and adiposity index of the WOKW rat were also determined to obtain more precise data about the obesity status of the WOKW rat. Comparative studies with several inbred strains also showed that the BMI of the WOKW rat is significantly higher (Klöting, unpublished data, 1999), indicating that the WOKW rat is obese. This could be further supported not only by the higher adiposity index but also by the fact that the WOKW rat has higher serum leptin, known to be an intriguing obesity-related quantitative trait. Obesity in the WOKW rat could favor the use of this model in comparison to the hereditary hypertriglyceridemic rat (hHTG). The hHTG rat is an established animal model of the metabolic syndrome; however, it is not obese, in contrast to the fully expressed syndrome in humans.[11]

Further characterization also showed that in comparison to DA rats, WOKW rats are hyperinsulinemic. Because it was previously reported that the WOKW rat manifests glucose intolerance, it could be suggested that insufficient insulin action rather than insulinopenia might be responsible for defective glucoregulation during the glucose tolerance test.

Regarding lipid phenotypes, the WOKW rat has higher serum triglycerides and lower HDL cholesterol than the DA rat, clearly indicating changes in terms of dyslipidemia, which is one of the main characteristics of the metabolic syndrome. Previously reported data on WOKW rats showed that serum triglyceride levels were about 3.3 mmol/L at an age interval of 18 to 26 weeks. Because in the present study 2.6 mmol/L serum triglyceride as a maximum value was measured (10-week-old rats), a further increase of triglycerides with age could be supposed in the WOKW rat. It would be consistent with previously reported

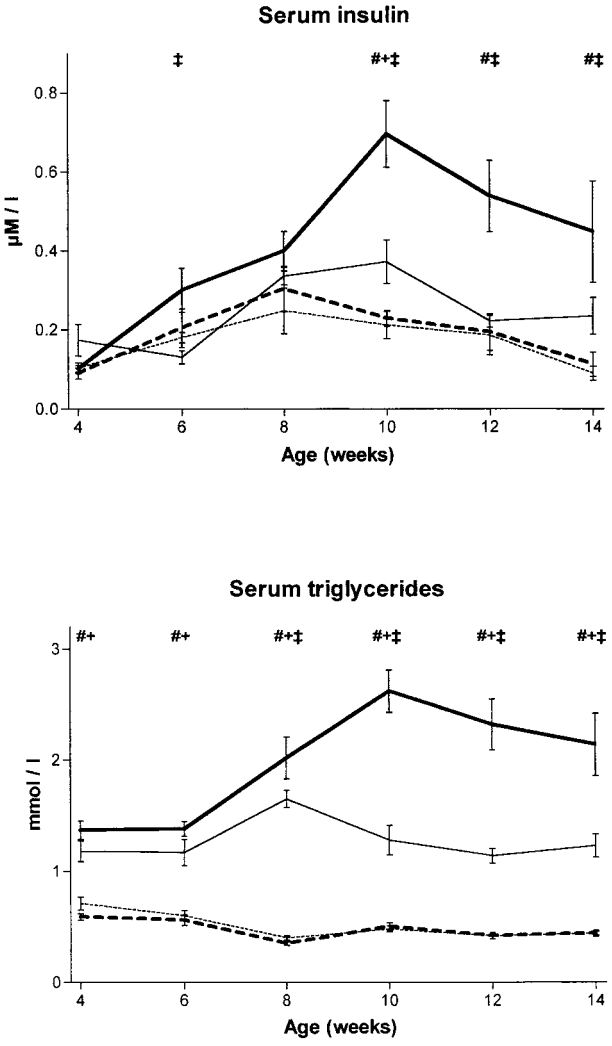

Fig 3. (A) Serum insulin and (B) triglycerides in WOKW and DA rats (males and females). See Fig 1.

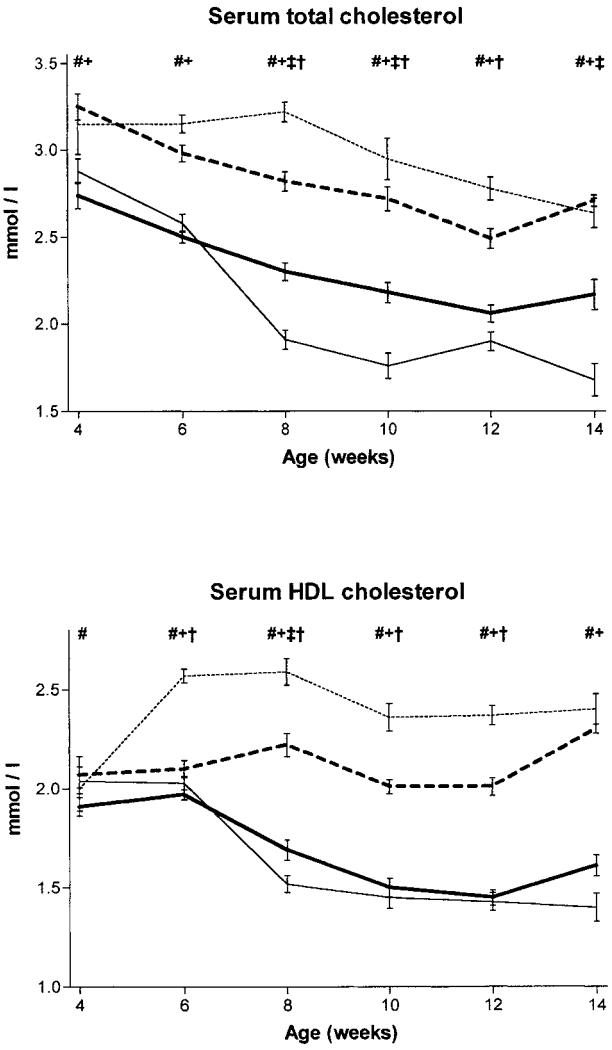

Fig 4. (A) Serum total cholesterol and (B) HDL cholesterol in WOKW and DA rats (males and females). See Fig 1.

findings indicating that serum triglycerides increase with age also in β and *fa/fa* rats or thioglucose-treated mice.[12,13] Although serum triglyceride levels seem to be lower in WOKW versus *fa/fa* rats, they are comparable with other established models of the metabolic syndrome and spontaneous hypertriglyceridemia such as hHTG rats or β-hypertriglyceridemic rats.[11,12]

The most dramatic increase of the body weight, BMI, serum leptin, serum insulin, and serum triglycerides in the WOKW rat in comparison to the DA rat was observed between 8 and 10 weeks of age for both sexes. However, at this age interval, the sex differences in the WOKW rat also indicate that the features of the metabolic syndrome in WOKW males are more pronounced versus WOKW females. These findings could be of great relevance in the choice of an appropriate age for phenotypic characterization of crossing populations in genetic studies using WOKW and DA strains, since it is known that a phenotypic difference between 2 parental strains is one of the most relevant factors determining the success of a search for genes with an impact on a certain phenotypic trait.[14]

The metabolic aberrations are moderate in the WOKW rat if compared, eg, with *fa/fa* rats representing genetically determined models of the metabolic syndrome, but are comparable to other established rat models such as the hHTG rat or the spontaneously hypertriglyceridemic inbred line of rats differentiated as β. However, sometimes it is difficult to distinguish between even the metabolic syndrome and type 2 diabetes mellitus, indicating that a different genetic background determines the expression of the specific mixture of metabolic aberrations. As stated by Shafrir,[9] a parallel situation exists in humans: not all patients with the metabolic syndrome manifest all elements of the cluster and there is an individual variation in the severity of complications, and therefore, even the models with milder types of the metabolic syndrome but determined polygenetically are of great relevance for pathophysiologic and genetic studies.

In conclusion, the results of our study provide further evidence for the availability of a new model manifesting the metabolic syndrome, since the WOKW rat is obese, dyslipidemic, hyperinsulinemic, and, as previously reported, moderately hypertensive and glucose-intolerant. The study determined that the manifestation of the features of the metabolic syndrome in the WOKW rat begins at age 8 to 10 weeks even without a special diet, which allows us to add this rat strain to animal models with a genetically determined metabolic syndrome. Furthermore, the study shows that regarding phenotypic differences, the DA rat might be a suitable control strain for a crossing study with the WOKW rat since, with respect to the lack of animal models with polygenic determined features of the metabolic syndrome, genetic analysis of the syndrome in the WOKW rat could shed more light on the genetic basis of this complex disorder.

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