Adiponectin values are unchanged during pregnancy in rats

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ABSTRACT. Adiponectin is believed to be a key factor in determining insulin sensitivity. In turn, insulin sensitivity is known to change from an enhanced state in early pregnancy to a reduced one in late pregnancy. A role for adiponectin in these changes has been proposed for mice but questioned for humans. We addressed this issue in rats by measuring adiponectin expression in both visceral and subcutaneous white adipose tissue, together with tissue content and release of the hormone in non-pregnant and in pregnant rats by days 8, 15, and 19 of pregnancy. Plasma concentration was also determined. No differences were found in any of the parameters measured between non-pregnant and pregnant rats at any time of pregnancy despite changes in white adipose tissue mass and insulin sensitivity. Adiponectin was also detected in cerebrospinal fluid at a concentration 1,000 times lower than in plasma, but again no differences were found between non-pregnant and pregnant animals. It is concluded that adiponectin does not play any role in regulating changes in insulin sensitivity during pregnancy in rats.

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INTRODUCTION

Traditional views of white adipose tissue (WAT) as a passive lipid storage organ have changed to a broader perspective, following the recognition that white adipocytes release a wide range of signalling molecules, the physiological significance of many of which is unknown (1-3). Adiponectin is expressed by adipocytes and has been detected in plasma of both rodents and humans (4, 5). Initially described as Acrp30 (4), it is also known as ApM1(6), AdipoQ (7) and GBP28 (8). It was linked to energy balance after the observation of both a diminished expression in WAT and decreased plasma levels in several obese rodent models and in humans (5, 7, 9). Elevation of plasma levels of adiponectin after direct administration of the molecule or of insulin sensitizers (such as thiazolidinediones) improves insulin sensitivity and reduces hyperglycemia in insulin-resistant animal models (10-12). In lipoatrophic mice, adiponectin reverses partially insulin resistance (13), and the protective role of the protein was confirmed with the adiponectin-null mice (14, 15). In a longitudinal study in the Rhesus monkey, a species that develops insulin resistance on aging, adiponectin plasma concentration declined in parallel with the appearance of insulin resistance (16). In humans, high levels of adiponectin are protective against the development of insulin resistance (17). Taken together all these observations have made adiponectin to be considered as a major modulator of insulin action.

During pregnancy, there is a continuous increase in body weight (18, 19), but the relative contribution to this increase from the maternal and fetal tissues varies throughout pregnancy. During the first two weeks of pregnancy, most of the body weight increase is due to maternal growth whereas in the last week maternal body weight stabilizes – or even decreases the day before labor – and fetal growth accounts for the increase in body weight (20, 21). The enhanced substrate demand of the fetuses is supplied by the mother primarily in the form of glucose or ketone bodies. To that end, an insulin-resistant state ensues, which allows enhanced hepatic gluconeogenesis and reduced glucose uptake in maternal muscle and WAT (22-25), thereby resulting in the maximum channeling of glucose to the fetuses. Therefore, pregnancy is a suitable model for studying natural changes in insulin sensitivity and the related endocrine factors.

Key-words: Cerebrospinal fluid, insulin sensitivity, plasma, white adipose tissue.
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609
Pregnancy leads to a reduction in plasma adiponectin in mice which agrees with a role as an insulin sensitizer (26, 27). None the less, in women adiponectin concentrations did not differ in third trimester pregnancy and post-partum samples which argues against such a role (28). We address this issue in another species, namely rats, hypothesizing differences between rodents and humans. Contrary to our expectations, we did not find any change in adiponectin related values with pregnancy in rats.

MATERIALS AND METHODS

Animals
Female Wistar rats weighing 180-200 g were housed at 22 °C in individual cages with water and food (A04 Panlab, Spain) freely available, and with a 12 h light/12 h dark cycle (lights on at 8:00 h). The stage of the estrus cycle was assessed daily to determine the reproductive status of the animals. In the morning of estrus, a female was housed with a male for 4 h. Mating was indicated by the presence of sperm in the vaginal smears. The day of mating was considered as day 0 of pregnancy. Virgins rats at diestrus II were used as representing day 0 of pregnancy, i.e. as non-pregnant rats. Animals were cared for and used in accordance with the principles of the Council of European Communities (86/609 EEC).

Sample collection
At days 8, 15 and 19 of pregnancy, animals were killed by decapitation after 10:00-12:00 h. Non-pregnant rats were killed at the same time interval. Just before sacrifice, cerebrospinal fluid (CSF) was obtained under halothane anesthesia by inserting a 23-gauge butterfly needle into the cisterna magna. Only samples without any blood contamination were processed. Trunk blood was collected at the time of decapitation. It was allowed to clot, centrifuged to obtain serum and stored at −80 °C until analysis. Parametrial, retroperitoneal and subcutaneous WAT depots from the left side of the animals were quickly removed, frozen in liquid N2 and stored at −80 °C until analysis. The corresponding right side WAT depots together with the perivascular, inguinal and perirenal depots were also removed and weighed.

Adiponectin tissue content
A fragment of parametrial WAT depot was homogenized in phosphate buffer (1 g in 1 ml), centrifuged at 3,400 g and the infranatant was assayed for the hormone.

Adiponectin release
Parametrial WAT was obtained in aseptic conditions, minced with scissors and a fragment of 250 mg incubated at 37 °C in 1 ml of Krebs-Ringer-Phosphate (pH 7.4) with glucose (1 mg/ml) and albumin (2%). After 8 h of incubation, the medium was recovered and stored at −80 °C until analysis.

mRNA detection
WAT samples were homogenized in Ultraspec reagent (Biotec Laboratories, USA), to obtain total cellular RNA, based on the single-step method of Chomczynski and Sacchi (29). The procedures used for the fractionation, blotting and hybridization of mRNAs together with a chemiluminescence detection protocol were carried out as described previously (30). In outline, RNAs were fractionated by agarose electrophoresis gel, transferred to a positively charged nylon membrane (Roche, Germany) by capillary blotting and cross-linked under UV light. Adiponectin’s mRNA was detected using a specific antisense oligonucleotide end-labelled 5’ with digoxigenin (Oswell, UK) as previously described (31). The membranes were incubated sequentially with the oligonucleotide and with an anti-digoxigenin FAB/alkaline phosphatase conjugate (Roche, Germany) and then processed essentially as in the protocols provided by the manufacturer. CDP-star® (Roche, Germany) was used as the chemiluminescence substrate. Signals were obtained by X-ray film exposure of the membrane (Hyperfilm ECL, Amersham Pharmacia Biotech, UK). Membranes were stripped and reprobed for 18S rRNA to adjust for any differences in the loading and transfer of RNA during blotting. 18S rRNA was detected using a 31-mer antisense oligonucleotide probe, as previously described (32).

Adiponectin detection
Serum adiponectin levels were measured with the mouse radioimmunoassay kit developed by Linco (USA), which is also suitable for rats. The limit of sensitivity was 1 ng/ml and the limit of linearity 100 ng/ml. The intra-assay coefficients of variation provided by the manufacturer were 3.73 and 4.11% for serum samples containing 3 and 8 µg/ml, respectively. The inter-assay coefficients of variation provided by the manufacturer were 8.24 and 6.56% for serum samples containing 3 and 8 µg/ml, respectively. According to the assay procedure, serum samples were diluted 1:1000 with assay buffer, prior to analysis. CSF was analyzed without dilution. Infranatants of tissue extracts were analyzed without any dilution whereas incubation medium of WAT fragments were diluted 100 times.

Insulin tolerance test (ITT)
Ratsfasted for 2 h were injected i.p. with insulin (human insulin, Lilly, Spain, 0.2 IU/100 g b.w.) between 12:00-13:00 h. Blood samples (50-60 µl) were collected by tail snip at 0 min (just before insulin injection) and at 15, 30, 45, 60 and 120 min after injection. Immediately, serum was separated and glucose analyzed by the GOD-POD method by using diagnostic reagent kit (Spinireact, Spain). The glucose disappearance rate (KITT) was calculated using the formula 0.693 (t1/2) (33). The plasma glucose half-life (t1/2) was calculated from the slope of the least-square analysis of the plasma glucose concentration during the linear decay phase. It has been previously shown a high degree of correlation between the ITT and clamp studies used to determine insulin sensitivity (34).

Statistical analysis
One-way analysis of variance (ANOVA) with time of pregnancy as a factor – non-pregnant rats representing day 0 of pregnancy - was used for comparing WAT depots mass, KITT, plasma and CSF adiponectin, the O.D. of the bands corresponding to adiponectin in Northern blots, adiponectin release in parametrial WAT incubations and adiponectin content in parametrial WAT depot. A Student-Newman-Keuls test was used for post-hoc comparisons. A two-way ANOVA for repeated measures was used for comparing body weight of pregnant and non-pregnant