Materials and Methods

Subjects

The subjects lived in Stockholm, Sweden and were since 1986 continuously recruited by local advertisement to study human adipose tissue function. A study on the impact of genetic polymorphism on lipolysis has been published on this cohort.[1] Individuals with diabetes treated with insulin, glitazones or glucagon like-peptide 1 analogues and subjects with severe chronic diseases were excluded. The cohort consisted of 301 men and 765 women (clinical data in Table 1). Sixty-one subjects were treated for type 2 diabetes with metformin and/or sulphonylurea, 45 for hypertension with beta-blockers and/or thiazides and/or 29 with drugs against hyperlipidemia. Each participant received a detailed description of the study and his/her informed written consent was obtained. Three individuals were minors (16-17 years) and for them informed consent was also obtained from the parents. The investigation was approved by the regional ethics board.

Procedures

The subjects were examined in the morning after an overnight fast. Height, weight and circumferences of waist and hip were determined. Total body fat was determined from a formula based on sex, BMI and age[2] which shows an excellent correlation with direct measures (demonstrated in Supplementary Figure 1 of [3]). After a 15-min rest in the supine position, a venous blood sample was obtained for determination of plasma TGs, HDL-C and total cholesterol by the hospital's routine clinical chemistry laboratory while FFAs were determined in serum using the NEFA C kit (Wako Chemicals, Neuss, Germany) as described.[4] For the latter, samples were stored at -

70 °C for a maximum of three weeks prior to analysis. Thereafter, a needle biopsy from the subcutaneous abdominal fat region was obtained under local anesthesia as described[5] Fat cells were isolated by collagenase treatment of adipose tissue and diluted fat cell suspensions (2%, vol/vol) were incubated for 2 hours at 37^{o}C in an albumin and glucose containing buffer (pH 7.4) as described.[6] The incubations were conducted in the absence (basal) or presence of increasing concentrations of the natural hormones noradrenaline, atrial natriuretic peptide or insulin. Glycerol release (an end product of lipolysis from different acylglycerols) was measured using a luminometric assay that is specific for this molecule.[7] Unlike for fatty acids there is no important re-utilization of glycerol by fat cells. Basal lipolysis is prominent in human white adipose tissue and not an artificial measure as discussed.[8-10] For example, the basal rate of glycerol release is constant for several hours in human subcutaneous WAT pieces incubated *in vitro*. Regarding catecholamines, noradrenaline was chosen instead of adrenaline because it more prominently reflects overall sympathetic activity and it is the main lipolysis regulator following physical activity whereas adrenaline above all is activated upon cold exposure and mental stress.[11] The latter stressors were regarded to be less relevant for this study. When insulin was used, 1 mU/l of adenosine deaminase was added to remove adenosine which is antilipolytic and may influence insulin action[12] Furthermore, 1 mmol/l of the phosphodiesterase sensitive cyclic AMP analogue 8-bromo cyclic AMP was added because insulin inhibits lipolysis through cyclic AMP hydrolysis[13] For basal lipolysis and lipolysis induced by noradrenaline or atrial natriuretic pepide, results were expressed at the maximum effective hormone concentrations (responsiveness). For all three hormones the lipolytic or antilipolytic sensitivity was also determined and expressed as pD_2, which is the negative 10 log molar value of the half maximum

effective hormone concentration. It was determined by logarithmic linearization of the concentration-response curves. According to classical pharmacology, responsiveness represents hormone action at distal steps from receptors and pD_2 reflects action at or a near the receptors, i.e. hormone sensitivity.[14] We did not analyze responsiveness for insulin because lipolysis was artificially stimulated by a pharmacological cyclic AMP analogue. Mean fat cell size and weight were determined exactly as described.[15, 16] In brief, the diameters of 100 cells were measured and used in well-established formulas to obtain mean size and weight. The accuracy of the method has been discussed in detail, including the counting of 100 cells instead of a larger number and that the fat cell diameters are normally distributed thus allowing the use of mean fat cell size and weight in the present study.[16] The number of fat cells incubated was determined by dividing lipid weight of the incubated sample by mean fat cell weight. There is no consensus on how to present values for glycerol release from adipose tissue or fat cells. As fat cell size was a co-factor in this study we à priori used expression per number of fat cells (glycerol levels corrected for 10^7 cells during 2 hrs) which enabled us to compare lipolysis with fat cell size in statistical analyses. In some cases glycerol release was also related to g tissue triglycerides and kg total fat mass. Only limited amounts of adipose tissue could be obtained by needle biopsy. Therefore it was not possible to perform a complete lipolysis investigation in all subjects although fat cell size and basal lipolysis were always measured. In addition atrial natriuretic peptide was only included in the analyses from 2007 and onwards when it became apparent that this hormone system might be physiologically relevant for human fat cell lipolysis as discussed.[17, 18] An overview of the fat cell data is shown in Table 1.

Statistics

Values are mean ± SD in text and tables and mean ± standard error of mean (SE) in figures. They were compared by analysis of covariance (ANCOVA) and single or multiple regression analysis as indicated in the text and table/figure legends.

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