Subcutaneous Adipocyte Lipolysis Contributes to Circulating Lipid Levels

Mikael Rydén, Peter Arner

Objective—Fatty acids released via fat cell lipolysis can affect circulating lipid levels. However, the contribution of different lipolysis measures in adipose tissue is unknown and was presently examined in isolated subcutaneous adipocytes.

Approach and Results—One thousand and sixty-six men and women were examined for lipolysis regulation in subcutaneous abdominal fat cells. Results were compared with fasting plasma levels of total cholesterol, high-density lipoprotein (HDL) cholesterol (HDL-C) and triglycerides. Spontaneous (basal) lipolysis and the effects of the major hormones stimulating (catecholamines and natriuretic peptides) and inhibiting lipolysis (insulin) were examined. Several statistically significant \((P<0.0001)\) correlations between the different lipolysis parameters and plasma lipids were observed. However, physiologically relevant correlations \((\text{adjusted } r^2>0.05)\) were only evident between basal or insulin-inhibited lipolysis and plasma triglycerides or HDL-C. Together, these lipolysis measures explained 14% of the variation in triglycerides or HDL-C, respectively. In comparison, a combination of established factors associated with variations in plasma lipids, that is, age; body mass index; waist circumference; waist-to-hip ratio; sex; nicotine use; fat cell volume; and pharmacotherapy against diabetes mellitus; hypertension; or hyperlipidemia explained 17% and 28%, respectively, of the variations in plasma triglycerides and HDL-C.

Conclusions—Subcutaneous fat cell lipolysis is an important independent contributor to interindividual variations in plasma lipids. High spontaneous lipolysis activity and resistance to the antilipolytic effect of insulin associate with elevated triglyceride and low HDL-C concentrations. Thus, although several other factors also play a role, subcutaneous adipose tissue may have a causal influence on dyslipidemia. (Arterioscler Thromb Vasc Biol. 2017;37:00-00. DOI: 10.1161/ATVBAHA.117.309759.)

Key Words: catecholamines • cholesterol • insulin • natriuretic peptides • triglycerides

It is now well established that elevated circulating cholesterol and triglycerides are important and independent risk factors for atherosclerotic cardiovascular disease.1–3 The regulation of plasma lipid levels is dependent on a variety of intrinsic and extrinsic factors.4–9 Bearing in mind the well-known role of fatty acids in dyslipidemia,10–11 one relevant endogenous factor could be fat cell lipolysis, a process that results in the release of free fatty acids (FFAs) to the circulation.4 FFAs are used for hepatic production of very-low-density lipoprotein triglycerides, which in turn may reduce high-density lipoprotein (HDL) cholesterol (HDL-C) levels by increasing the transfer of triglycerides to HDL.7,12,13 These triglyceride-rich HDL particles are subsequently hydrolyzed by hepatic lipase14 leading to formation of remnant HDL.15 Consequently, a high lipolysis rate might result in hypertriglyceridemia and low HDL-C levels.7,12,13 The release and storage of adipose FFAs is determined by the turnover of fat cell triglycerides. We recently developed a method to determine adipocyte lipid turnover in free living humans16 and could demonstrate that common and genetic forms of dyslipidemia were linked to altered triglyceride turnover, which in turn was proportional to subcutaneous adipocyte lipolysis activity.16,17 Given that subcutaneous adipose tissue constitutes the by far largest fat depot, these studies suggest that fat cell lipolysis may, at least in part, affect circulating lipid levels.

Lipolysis in human fat cells has a unique regulation compared with rodents.18–20 Thus, human adipocytes display a substantial spontaneous (basal) lipolytic activity and catecholamines and natriuretic peptides are the only hormones with a pronounced prolipolytic activity. The effects of natriuretic peptides in adipocytes of humans and primates contrast with rodent cells where these agents display no effect. In both humans and rodents, insulin is the major antilipolytic hormone.

Despite the well-known impact of lipolysis on circulating lipids in man, surprisingly few studies have directly examined fat cell lipolysis. Thus, most are small cohort studies on subcutaneous fat cells in hereditary forms of dyslipidemia, that is, endogenous hypertriglyceridemia or familial combined
hyperlipidemia.21–26 These investigations have reported normal basal but blunted pro- and antilipolytic effects of catecholamines and insulin, respectively. One study investigated visceral fat cell lipolysis in 45 subjects who were not selected according to circulating lipid levels.27 An inverse relationship was observed between catecholamine-stimulated lipolysis and HDL-C but not with triglyceride levels.

Although the few published studies discussed above suggest that adipocyte lipolysis might influence circulating lipid levels, several unanswered questions remain. How much does subcutaneous fat cell lipolysis contribute? What are the relative roles of basal lipolysis and lipolysis induced by different hormone systems? What is the influence of important intrinsic and extrinsic factors such as age, sex, body mass index, body fat distribution, nicotine use, or concomitant medication? To answer these questions, we conducted a large study (>1000 subjects) on abdominal subcutaneous fat cells examining the possible relationships between basal and hormone-regulated lipolysis on the one hand and fasting plasma levels of total cholesterol, HDL-C, and triglycerides, on the other hand.

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Results

We first investigated the correlations between each of the fat cell lipolysis and plasma lipid measures by single linear regression (Table 1). The relationship between several circulating lipid and lipolysis measures were statistically significant. In many cases, however, the level of correlation as determined by adjusted $r^2$ values was low ($<0.05$) and was not considered to be physiologically relevant. On the contrary, the correlations between antilipolytic insulin sensitivity or basal lipolysis and plasma triglycerides or HDL-C were stronger (adjusted $r^2=0.05–0.11$) and, therefore, selected for further investigations. Basal lipolysis was negatively correlated with HDL-C and positively with triglycerides (Figure 1A), whereas the opposite relationships were observed for antilipolytic insulin sensitivity (ie, $pD2$; Figure 1B). When dividing the subjects into tertiles according to HDL-C and triglyceride levels, basal lipolysis and antilipolytic insulin sensitivity were significantly different between the 2 extreme groups (ie, high HDL-C/low triglyceride versus low HDL-C/high triglyceride; Figure 1C and 1D). The cohort included obese (n=599) and nonobese (n=467) subjects. The relationships between HDL-C and triglycerides for basal lipolysis were stronger in nonobese subjects (HDL-C: nonobese $r=-0.27, P<0.0001$; obese $r=-0.10, P=0.012$ and triglycerides: nonobese $r=0.27, P<0.0001$; obese $r=0.12, P=0.0035$). For antilipolytic insulin sensitivity, the corresponding numbers were for HDL-C: nonobese $r=0.17, P=0.044$; obese $r=0.14, P=0.017$ and triglyceride: nonobese $r=-0.31, P=0.0003$; obese $r=-0.21, P=0.0002$. Altogether, these results suggest that basal lipolysis is better associated with HDL-C/triglycerides in nonobese compared with obese subjects. In contrast, antilipolytic insulin sensitivity is strongly associated with triglycerides in both nonobese and obese subjects. Twenty-nine individuals were on treatment against antihyperlipidemia, and 61 subjects against type 2 diabetes mellitus. These groups were too small to allow a reliable subgroup analysis. Nevertheless, removing these individuals from the analyses provided similar $r$ and $P$ values as in Table 1 (data not shown). Moreover, there was no sex interaction in the relationships between basal lipolysis or antilipolytic insulin sensitivity and HDL-C or triglycerides ($F=0.1–2.4; P=0.12–0.76$).

We next investigated whether the relationships between antilipolytic insulin sensitivity/basal lipolysis and triglyceride/ HDL-C remained after correction for intrinsic/extrinsic factors known to influence circulating lipid levels (Table 2). This demonstrated that the lipolysis/plasma lipid relationships were still highly significant ($r<0.0001$) after correction for sex; age; body mass index; waist circumference; waist-to-hip ratio; nicotine use; treatment of type 2 diabetes mellitus, hypertension, or hyperlipidemia; and fat cell size. As expected, several of the cofactors correlated significantly with the plasma lipid levels in Table 2. On the contrary, most of these correlations were weak (adjusted $r<0.05$). For HDL-C, the only relevant relationships were observed with fat cell size and waist-to-hip ratio (adjusted $r=0.14–0.16$). For plasma triglycerides, these stronger associations were instead found with body mass index and waist-to-hip ratio (adjusted $r=0.07–0.14$).

Using different regression models, we also investigated the influence on plasma lipids of combining the cofactors in Table 2 and the 2 lipolysis measures (Table 3). Adjusted $r^2$ for

### Table 1. Correlation Between Subcutaneous Adipose Lipolysis and Fasting Plasma Lipid Levels

<table>
<thead>
<tr>
<th>Adipose Parameter</th>
<th>Total Cholesterol</th>
<th>HDL-C</th>
<th>Triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r$ (Adjusted $r^2$)</td>
<td>$P$ Value</td>
<td>$r$ (Adjusted $r^2$)</td>
</tr>
<tr>
<td>Basal lipolysis</td>
<td>0.09 (0.007)</td>
<td>0.004</td>
<td>-0.25* (0.06)</td>
</tr>
<tr>
<td>Maximum noradrenaline-induced lipolysis</td>
<td>0.008 (0)</td>
<td>0.80</td>
<td>-0.20 (0.037)</td>
</tr>
<tr>
<td>Noradrenaline lipolytic sensitivity</td>
<td>-0.07 (0.004)</td>
<td>0.054</td>
<td>0.02 (&lt;0.001)</td>
</tr>
<tr>
<td>Maximum atrial natriuretic peptide-induced lipolysis</td>
<td>0.02 (0)</td>
<td>0.79</td>
<td>-0.13 (0.01)</td>
</tr>
<tr>
<td>Atrial natriuretic peptide lipolytic sensitivity</td>
<td>0.05 (&lt;0.001)</td>
<td>0.59</td>
<td>0.006 (&lt;0.001)</td>
</tr>
<tr>
<td>Antilipolytic insulin sensitivity</td>
<td>0.20 (0.036)</td>
<td>&lt;0.0001</td>
<td>0.30* (0.09)</td>
</tr>
</tbody>
</table>

Linear regression was used. Triglyceride values were log$_{10}$ transformed. HDL-C indicates high-density lipoprotein cholesterol.
antilipolytic insulin sensitivity and basal lipolysis put together was 0.14 for both triglycerides and HDL-C. In this model, each of the lipolysis measures contributed significantly ($P < 0.0001$) to the variation in lipid levels. All cofactors combined but without lipolysis measures yielded adjusted $r^2$ of 0.17 for triglycerides and 0.28 for HDL-C. These values increased to 0.29 and 0.36, respectively, when basal lipolysis and antilipolytic insulin sensitivity were included in the model.

We finally made some further studies of basal lipolysis (Figure 1). The rate in isolated fat cells was strongly and positively correlated with the spontaneous rate of lipolysis in pieces of adipose tissue (Figure 2A). Basal rate of lipolysis and pD$_2$ for insulin were negatively correlated (Figure 2B). The impact of basal lipolysis on fasting serum FFA levels was also analyzed. When subjects were divided into tertiles according to circulating FFA levels, the basal rate of lipolysis expressed per fat cell number (Figure 2C) or corrected for total body fat mass (Figure 2D) was significantly higher in the highest compared with the lowest tertile. Similar results were observed when basal lipolysis was expressed per tissue fat weight (24% higher in the highest tertile; $P = 0.0045$; graph not shown). There was a linear relationship between total body fat mass and dyslipidemia (Table 1).

### Table 2. Relationship Between Fasting Plasma Lipid Levels and Lipolysis in Subcutaneous Fat Cells Corrected for Cofactors

<table>
<thead>
<tr>
<th>Cofactor</th>
<th>Triglycerides</th>
<th>HDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal Lipolysis</td>
<td>Antilipolytic Insulin Sensitivity</td>
</tr>
<tr>
<td>Sex</td>
<td>Partial $r$</td>
<td>$P$ Value</td>
</tr>
<tr>
<td>Age</td>
<td>0.22</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>BMI</td>
<td>0.23</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>0.15</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.16</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>Nicotine use</td>
<td>0.22</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>Type 2 diabetes mellitus treatment</td>
<td>0.23</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>Antihypertensive treatment</td>
<td>0.23</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>Hyperlipidemia treatment</td>
<td>0.22</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>Fat cell volume</td>
<td>0.13</td>
<td>$0.0001$</td>
</tr>
<tr>
<td>HDL-C or triglyceride</td>
<td>0.13</td>
<td>$&lt;0.0001$</td>
</tr>
</tbody>
</table>

Multiple regression was used. Triglyceride values were log$_{10}$ transformed. BMI indicates body mass index; and HDL-C, high-density lipoprotein cholesterol.
fat cell lipolysis and circulating FFAs ($r=0.19$; $P<0.0001$; Figure 2E, top). Although this association was more evident in men ($r=0.34$; $P<0.0001$; Figure 2E, bottom) than in women ($r=0.15$; $P<0.0001$; Figure 2E, middle), there was no sex interaction ($F=2.6$; $P=0.11$). None of these relationships were observed for insulin pD2 (graphs not shown).

**Discussion**

This study sheds new light on the role of subcutaneous fat cell lipolysis in influencing circulating lipid levels. When examining several relevant measures of lipolysis (basal activity or effect of different hormones), only the basal rate and the antilipolytic effect of insulin displayed clinically relevant relationships. Furthermore, the influence of adipocyte lipolysis was only important for plasma HDL-C and triglycerides. These results indicate that resistance to the antilipolytic effect of insulin and a high rate of basal lipolysis are associated with low HDL-C and high triglycerides levels. This dyslipidemic pattern confers increased risk for atherosclerotic cardiovascular disease. Importantly, the observed relationships were independent of classical risk factors influencing plasma lipids such as sex; age; body mass index; body shape; use of nicotine; treatment of hypertension, diabetes mellitus, or hyperlipidemia; and fat cell size.

Is increased lipolytic activity in fat cells clinically important for dyslipidemia? Our results would suggest so. When put together, the 2 lipolytic measures contributed independently and explained 14% of the variations in plasma triglycerides or HDL-C. The influence of lipolysis can be compared with that of the combined risk factors listed in Table 2. Together, they explained only 17% of the variation in triglycerides and 28% of that in HDL-C. When combined with the lipolysis measures, the influence rose to 29% and 36%, respectively. Thus, the impact of subcutaneous adipocyte lipolysis on plasma lipid levels can be regarded as physiologically relevant.

Fat cell lipolysis is subjected to regional variations as reviewed.28,29 The lipolytic activity is higher in visceral than the presently examined subcutaneous depot. However, subcutaneous adipose tissue is by far the body’s largest fat depot, and an important mass effect of its lipolytic action is, therefore, likely. This notion is supported by studies of Jensen30 and his

### Table 3. Influence of Cofactors and Lipolysis Parameters Put Together on Plasma Lipid Levels

<table>
<thead>
<tr>
<th>Models</th>
<th>HDL-C</th>
<th>Triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adjusted $r^2$</td>
<td>$P$ Value</td>
</tr>
<tr>
<td>Antilipolytic insulin sensitivity and basal lipolysis put together</td>
<td>0.14 &lt;0.0001</td>
<td>0.14 &lt;0.0001</td>
</tr>
<tr>
<td>All cofactors in Table 2 put together</td>
<td>0.28 &lt;0.0001</td>
<td>0.17 &lt;0.0001</td>
</tr>
<tr>
<td>All cofactors plus antilipolytic insulin sensitivity and basal lipolysis put together</td>
<td>0.36 &lt;0.0001</td>
<td>0.29 &lt;0.0001</td>
</tr>
</tbody>
</table>

Multiple regression analysis was performed. Plasma triglyceride values were log$_{10}$ transformed. HDL-C indicates high-density lipoprotein cholesterol.

![Figure 2](http://atvb.ahajournals.org/)

Figure 2. Findings with basal lipolysis. Relationship between lipolysis (determined by glycerol release in isolated fat cells and corrected for fat cell number) and (A) pieces of adipose tissue, (B) insulin pD2, and (C) circulating free fatty acid (FFA) levels subdivided into tertiles. (D) There was a similar relationship between basal lipolysis corrected for total body fat mass and circulating FFA levels subdivided into tertiles. (E) Basal lipolysis corrected for total fat mass correlated with circulating FFA levels in the entire cohort (top), in women (middle) and men (bottom). Linear regression analysis was used in (A), (B), and (E). $r$ and $P$ values are indicated. Data in (C) and (D) were compared by ANCOVA, overall $P$ value is given.
colleagues, demonstrating that subcutaneous WAT is a much more important contributor to hepatic fatty acids than visceral fat. Our present findings on serum FFAs are in line with these results showing that high levels were strongly correlated with a high rate of basal lipolysis in subcutaneous fat cells irrespec-
tively of how basal lipolysis was expressed. It would nevertheless have been interesting to compare the results obtained herein with lipolysis measures in visceral fat cells from the same individuals. Unfortunately, such a study would be difficult to perform. Visceral fat can only be obtained in connection with general surgery, and it would be impossible to standardize patient selection and surgical or anesthetic procedures for a long period of time. In our investigation, we used the same methods that were performed by the same staff throughout the study.

Lipolysis was measured in isolated fat cells, and it might be argued that at least basal lipolysis in this cell preparation does not measure a clinically relevant form of lipolysis. This is, however, unlikely because we observed a strong correlation between the rate of basal lipolysis in isolated fat cells and adipose tissue pieces ex vivo. Furthermore, lipolysis was determined in vitro, which may differ from that observed in vivo. Methods to assess the latter involve cumbersome microdialysis or tracer catheterization methods that are not suitable for the present type of large-scale study. Importantly though, smaller studies have observed similar results when comparing subcutaneous adipose tissue lipolysis in vivo and in vitro.31,32 Finally, we used a pharmacological evaluation of concentration–response curves instead of measuring lipolysis at a particular hormone concentration representative of the circulating level. There are several reasons for this approach. First, hormone levels in blood vary considerably between the resting state and on different challenges. Second, the important concentrations for this study are those at the cellular levels. For noradrenaline, they are unknown but probably much higher than in blood because the hormone is released through nerve endings in adipose tissue. For insulin, the concentration levels are much lower in blood than in the circulation both in the fasting and fed states.3 In any case, the pD2 values for the different hormones (Table 4) suggest that the half-maximum effective concentrations in our experiments were within the physiological range.

The inherent epidemiological design of this study does not allow firm conclusions on how lipolysis contributes to dys-
lipidemia at the molecular level. Nevertheless, some mechan-
istic explanations can be hypothesized. It is obvious that variations in specific lipolysis-regulating factors are differen-
tially linked to HDL-C/triglycerides. These involve changes independent of regulating hormone receptors (basal lipolysis) or events at or near the insulin receptor (insulin pD2). In contrast, hormone-stimulated lipolysis (mediated either by noradrenaline or ANP) seems to be of less importance. This suggests that increased lipolysis in the resting (basal) and postprandial (insulin-mediated inhibition) states are more relevant in explaining variations in circulating lipid levels than lipolysis induced by physical activity or mental stress (ie, catecholamines). As discussed above, increased lipolysis and FFAs can affect dyslipidemia through direct effects on hepatic very-low-density lipoprotein assembly and secretion.5–11 However, indirect effects may also play a role, for instance,

### Table 4. Cohort Characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean±SD or Number</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (males/females)</td>
<td>301/765</td>
<td>…</td>
</tr>
<tr>
<td>Age, y (n=1066)</td>
<td>42±12</td>
<td>16–79</td>
</tr>
<tr>
<td>BMI, kg/m² (n=1066)</td>
<td>33±8.5</td>
<td>18–63</td>
</tr>
<tr>
<td>Obesity, yes/no</td>
<td>599/467</td>
<td>…</td>
</tr>
<tr>
<td>Body fat, % (n=1066)</td>
<td>40±14</td>
<td>8–86</td>
</tr>
<tr>
<td>Waist circumference, cm (n=1032)</td>
<td>106±20</td>
<td>65–154</td>
</tr>
<tr>
<td>Waist-to-hip ratio (n=1031)</td>
<td>0.94±0.09</td>
<td>0.70–1.21</td>
</tr>
<tr>
<td>P-total cholesterol, mmol/L (n=1065)</td>
<td>5.1±1.2</td>
<td>2.5–12.8</td>
</tr>
<tr>
<td>P-HDL-C, mmol/L (n=1034)</td>
<td>1.3±0.4</td>
<td>0.5–2.9</td>
</tr>
<tr>
<td>P-triglycerides, mmol/L (n=1062)</td>
<td>1.5±1.4</td>
<td>0.0–21.7</td>
</tr>
<tr>
<td>S-fatty acids, mmol/L (n=780)</td>
<td>0.66±0.24</td>
<td>0.07–2.0</td>
</tr>
<tr>
<td>fP-glucose, mmol/L (n=1043)</td>
<td>5.5±1.3</td>
<td>3.4–20.9</td>
</tr>
<tr>
<td>HbA1c, mmol/mol (n=298)</td>
<td>39±9</td>
<td>20–97</td>
</tr>
<tr>
<td>Nicotine use, yes/no (n=1040)</td>
<td>269/771</td>
<td>…</td>
</tr>
<tr>
<td>Fat cell volume, pl (n=1049)</td>
<td>687±252</td>
<td>72–1452</td>
</tr>
<tr>
<td>Basal lipolysis* (n=1052)</td>
<td>6.4±5.8</td>
<td>0.0–38.6</td>
</tr>
<tr>
<td>Maximum noradrenaline-induced lipolysis* (n=1032)</td>
<td>16.1±10.9</td>
<td>1.3–75.9</td>
</tr>
<tr>
<td>Noradrenaline sensitivity, pD2* (n=722)</td>
<td>7.9±1.3</td>
<td>1.1–12.0</td>
</tr>
<tr>
<td>Maximum atrial natriuretic peptide–induced lipolysis* (n=132)</td>
<td>12.9±7.3</td>
<td>0.4–41.0</td>
</tr>
<tr>
<td>Atrial natriuretic peptide sensitivity, pD2* (n=130)</td>
<td>9.1±1.6</td>
<td>3.5–14.7</td>
</tr>
<tr>
<td>Antilipolytic insulin sensitivity, pD2* (n=447)</td>
<td>14.2±1.6</td>
<td>6.5–17.0</td>
</tr>
<tr>
<td>Adipose tissue spontaneous lipolysis* (n=634)</td>
<td>5.3±3.1</td>
<td>0.4–18.6</td>
</tr>
<tr>
<td>Medication against T2D/hyperlipidemia/ hypertension</td>
<td>61±29/45</td>
<td>…</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; HDL-C, high-density lipoprotein cholesterol; P, fasting plasma; S, fasting serum; and T2D, type 2 diabetes mellitus.

Values are for isolated fat cells or adipose tissue and expressed as mmol of glycerol/2 h/10⁷ fat cells or negative log10 molar concentration for half-maximum hormone effect (pD2).

FFA-mediated insulin resistance35 and altered cholesteryl ester transfer protein activity.36

In summary, this study suggests that lipolysis in subcutaneous fat cells is an important and independent contributor to variations in plasma triglycerides and HDL-C levels. This relationship pertains specifically to spontaneous (basal) and insulin-inhibited lipolysis.

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Disclosures

None.

References


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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. 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 which shows an excellent correlation with direct measures (demonstrated in Supplementary Figure 1 of ). 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. 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. 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°C in an albumin and glucose containing buffer (pH 7.4) as described. 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. 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. 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. 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. 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. For basal lipolysis and lipolysis induced by noradrenaline or atrial natriuretic peptide, 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 pD₂, 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 pD₂ reflects action at or 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 10⁷ 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.

References

8. Langin D. Adipose tissue lipolysis as a metabolic pathway to define pharmacological strategies against obesity and the metabolic syndrome. *Pharmacol Res*. 2006;53:482-491

