Adipose tissue autophagy related gene expression is associated with glucometabolic status in human obesity

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ABSTRACT

Adipose tissue autophagy (AT) is associated with human obesity and increased metabolic risk. Recent findings establish a role for autophagy in lipid metabolism. Here, we compared the expression of autophagy-related and lipolysis genes in human abdominal subcutaneous AT (SCAT) in overweight/obese subjects (n = 17) with or without impaired glucose tolerance in comparison with lean normal glucose tolerant individuals (n = 9), and investigated the association between AT autophagy and lipolysis. Human multipotent adipose-derived stem cells (hMADS) were used to investigate the effect of pharmacological HSL inhibition on changes in the autophagic flux. The expression of autophagy-related genes (ATG) 5, 7 and 12 in SCAT was significantly higher (p = 0.043, p = 0.015, p = 0.004, respectively) in overweight/obese compared to lean men, while expression of the classical lipases HSL (p = 0.092) and ATGL (p = 0.084) tended to be lower. ATG12 mRNA was positively correlated with BMI (r = 0.407, p = 0.039). ATG7 mRNA correlated positively with waist/hip ratio (WHR) (r = 0.420, p = 0.041), 2 h glucose concentration (r = 0.488, p = 0.011) and insulin (r = 0.419, p = 0.033). Multiple linear regressions revealed that ATG7 gene expression was positively related to 2 h glucose, independent of BMI, WHR and insulin. Gene expression interaction analysis showed that ATG7 mRNA negatively correlated with HSL (p < 0.01) and ATGL mRNA expression (p < 0.01). In line, treatment of differentiated hMADS with an HSL inhibitor increased LC3 accumulation, a marker of increased autophagic flux. Collectively, the present study demonstrated that a low expression of classical lipases in abdominal SCAT is accompanied by an increased expression of ATGs in overweight/obese subjects, which seems to be mainly related to glucose tolerance.

KEYWORDS

Adipose tissue; adipocyte; autophagy; lipolysis; obesity; glucometabolic status

Introduction

Excessive lipid accumulation in obese adipose tissue (AT) is partly the result of impaired lipid turnover, which is determined by lipid uptake, lipogenesis and lipolysis. Adipose tissue lipolysis is mainly mediated through the sequential removal of fatty acids from the glycerol backbone of triacylglycerol by the consecutive action of adipose tissue triglyceride lipase (ATGL), hormone sensitive lipase (HSL) and monoglyceride lipase (MGL). Following lipolysis, the liberated fatty acids are directed towards oxidation in the mitochondria of adipocytes, released into the circulation to be used by peripheral tissues, or re-esterified into triacylglycerol. Impaired regulation of AT lipolysis may contribute to the development of overweight and obesity, resulting in systemic lipid overflow which may subsequently lead to ectopic accumulation of lipid intermediates and insulin resistance.

Recently, autophagy has emerged as an important player in obesity and related metabolic disorders. Autophagy is a homeostatic mechanism functioning as a disposal system that degrades large intracellular organelles or protein aggregates to change cellular structure during differentiation, to generate essential nutrients in times of energy deprivation, and to prevent accumulation of damaged proteins and organelles. It has been shown that autophagy is involved in adipogenesis and fatty acid oxidation, at least in murine adipocytes. Autophagy markers have been reported to be upregulated in AT of both obese mice and humans in most but not all studies. Indeed, the autophagic flux is most likely upregulated in obese AT which has been

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Supplemental data for this article can be accessed on the publisher’s website.

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suggested to reflect insulin resistance. It is well known that insulin inhibits autophagy through activation of mTORC1. In line, insulin resistance will attenuate mTOR activity and consequently activate the autophagy pathway. In accordance, autophagy was enhanced in adipocytes from type 2 diabetic subjects with an attenuated mTOR-S6K1. Furthermore, autophagy activation is more pronounced in visceral than in subcutaneous AT, which is particularly evident in insulin resistant obese patients with elevated intra-abdominal fat mass. Moreover, the increased expression of autophagy-related genes correlated with the degree of obesity, visceral fat mass, and adipocyte hypertrophy. In addition, the activation of autophagy coincides with the development of insulin resistance and impaired glucose metabolism in rodents. Therefore, elevated autophagy may represent a protective, compensatory mechanism against obesity-related AT dysfunction. 

In the present study, we investigated the expression of autophagy-related genes (ATG) and classical lipolysis genes in abdominal subcutaneous AT (SCAT) from subjects with a wide range of BMI and different glucometabolic status. We examined the relation between gene expression and phenotype, and also the interaction between autophagy-related genes and classical lipolysis genes. Finally, experiments using differentiated human multipotent adipose-derived stem cells (hMADS) were performed for mechanistic deepening.

**Results**

**Subjects’ characteristics**

Clinical characteristics of the participants are summarized in Table 1. Briefly, 9 male lean participants and 17 overweight/obese participants (12 male/5 female), were enrolled. The overweight/obese subjects had a significantly higher body weight, BMI, waist, hip, 2 h glucose, fasting insulin concentration and HOMA-IR compared to the lean.

**Autophagy-related gene expression is increased in overweight/obese subcutaneous AT**

Gene expression of ATG5, ATG7, ATG12, involved in autophagosome formation, was significantly higher in the subcutaneous AT of overweight/obese compared to lean subjects (p = 0.043, p = 0.015, p = 0.004, respectively; Fig. 1). This was accompanied by a trend towards a lower expression of the classical lipolysis genes HSL and ATGL in the overweight/obese group (P = 0.092, P = 0.084, respectively; Fig. 1). Expression of ATG12 mRNA expression was positively correlated with BMI (r = 0.407, P = 0.039). Expression of ATG7 mRNA was positively associated with waist/hip ratio (WHR) (r = 0.420, P = 0.041), 2 h glucose (r = 0.488, p = 0.011), fasting insulin (r = 0.419, p = 0.033), HOMA-IR (r = 0.407, p = 0.039), and a trend for correlation with BMI (r = 0.380, p = 0.055; Table 2). Backward stepwise elimination in multiple linear regression (dependent: ATG7; independent: BMI, WHR, 2 h glucose, insulin) revealed that ATG7 gene expression was significantly related to 2 h glucose (r = 0.489, p = 0.012), independent of WHR, insulin and BMI.

**Gene expression interaction analysis**

The expression of the classical lipolysis genes ATGL and HSL was negatively correlated with ATG7 (r = -0.537, P = 0.005; r = -0.537, P = 0.005, respectively; Fig. 2). To further evaluate the relationship between markers of autophagy and classical lipolysis, we performed correlation clustering. This method assumes that genes, for which the expression values are correlated, are co-regulated and

![Figure 1. mRNA expression of the autophagy and classical lipolysis markers in human subcutaneous AT of overweight/obese (n = 17; black bars) compared to lean (n = 9; white bars) subjects. Data are presented as mean ± SEM. *p < 0.05, **p < 0.01, independent samples t-test compared to lean subjects.](image-url)
belong to the same process or to interacting processes. Only correlations between genes with \( P \leq 0.01 \) were regarded relevant. All studied autophagy-related genes were positively clustered with RAB7A, ATG12 and ATG7 as major nodes (Fig. 2). Also, the two classical lipolysis genes were shown to interact, but there was a significant negative interaction between these genes and the autophagy cluster through a negative correlation with ATG7.

**Table 2.** Pearson’s correlation coefficients for autophagy-related gene expression and clinical characteristics.

| Gene symbol | TFEB | ULK1 | ULK2 | BECN-1 | ATG5 | ATG12 | ATG7 | LC3A | LC3B | LIPA | ATGL | HSL |
|-------------|------|------|------|--------|------|-------|------|------|------|------|------|-----|
| BMI \( \text{kg/m}^2 \) | 0.036 | 0.270 | 0.096 | 0.005  | 0.324 | 0.407  | 0.380 | 0.266 | 0.250 | 0.102 | −0.390 | −0.422 |
| WHR         | 0.104 | 0.014 | 0.029 | 0.257  | −0.010 | 0.134  | 0.420  | 0.003 | 0.311 | 0.304 | −0.310 | −0.318 |
| Fasting glucose \( \text{mM} \) | −0.060 | −0.063 | −0.186 | 0.176  | 0.364  | 0.082  | 0.193  | −0.111 | 0.277  | −0.056 | −0.264 | −0.311 |
| 2 h glucose \( \text{mM} \) | 0.104 | −0.261 | −0.062 | 0.342  | 0.060  | −0.148 | 0.488  | −0.004 | 0.063  | 0.103  | −0.409  | −0.534 |
| Insulin \( \text{mU/L} \) | 0.119 | 0.016 | −0.013 | 0.020  | 0.194  | 0.228  | 0.419  | 0.127 | 0.146  | 0.258  | −0.248  | −0.293 |
| HOMA-IR     | 0.075 | −0.027 | −0.093 | 0.057  | 0.263  | 0.207  | 0.407  | 0.051 | 0.188  | 0.213  | −0.297  | −0.347 |
| Fat cell size \( \mu \text{m} \) | −0.319 | −0.087 | −0.314 | −0.303 | 0.110  | 0.178  | 0.059  | −0.267 | 0.019  | 0.088  | −0.350  | −0.325 |

\( ^* p \leq 0.05, \) Pearson’s correlation

**Lipase inhibition elevated autophagic flux in human adipocytes**

To provide mechanistic insight into the inverse association between classical lipases and autophagy-markers, we incubated differentiated hMADS with an HSL inhibitor and evaluated the effect on autophagic flux using Western blotting. As shown in Fig. 3, differentiated hMADS treated with an HSL inhibitor in combination with Bafilomycin showed an elevated LC3II/LC3I ratio compared to cells only treated with Bafilomycin. The change in P62 was not significant. Together these data indicate an increased autophagic flux following lipase inhibition.

**Discussion**

The present study investigated lipase and autophagy-related gene expression in abdominal subcutaneous AT of subjects with a wide range of BMI and glucometabolic status. We demonstrate that ATG5, ATG7 and ATG12, which are important for the conjugation cascade of the autophagosome, are upregulated in overweight/obese compared to lean subjects. This was accompanied by an attenuated expression of the classical lipases ATGL and HSL. Correlation analysis revealed that both autophagy and lipolysis are interacting in an opposite manner, with ATG7 as a possible linking node. Moreover, ATG7 gene expression was correlated with 2 h glucose, linking adipose tissue autophagy to whole-body glucose metabolism. The finding that ATG7 also negatively correlated

**Figure 2.** Correlation among ATGs and classical lipolysis genes. ATGs positively correlated with each other, all lines mean \( p \leq 0.01 \). Positive correlation, solid lines; negative correlation, dash lines.

**Figure 3.** (A) Representative LC3, P62 and GAPDH western blots of differentiated human adipocytes treated with or without Bafilomycin (Baf) and with or without HSL inhibitor (HSLinh) for 48 hours. (B) Quantification of LC3 II/I ratio and (C) P62 as markers of autophagic flux, \( * p < 0.05 \), paired t-test. Data are presented as mean ± SEM.
with HSL and ATGL was in line with our cell experiments, in which the blockage of the classical pathway led to an increase of the autophagic flux in differentiated human adipocytes.

Although autophagy was thought to be mainly regulated by posttranscriptional mechanisms, more recent studies show that transcriptional dysregulation of adipose tissue autophagy is currently emerging in human obesity. In the present study, we found that ATG5, ATG7 and ATG12 mRNA levels are significantly higher in the overweight/obese compared to lean age-matched subjects. These data indicate that mainly the autophagic conjugation cascade might be elevated in obese subcutaneous adipose tissue. ATG12 conjugates with ATG5 through the concerted action of ATG7 (an E1-like ubiquitin-activating enzyme) and ATG10 (an E2-like ubiquitin-conjugating enzyme), forming the ATG12-ATG5-ATG16L complex, which then participates in LC3-phosphatidylethanolamine conjugation. After processing, the lipid-conjugated LC3, which is localized to the autophagosomal membranes, participates in the formation and elongation of autophagosomes. In line with our results, several studies have shown that gene expression of major autophagy-related genes (ATGs) is higher in AT of obese rodents and humans. Kovsan and colleagues have shown that ATG5, ATG12 and LC3 mRNA expression is up-regulated in human subcutaneous and visceral AT in both non-diabetic and diabetic obese subjects. In contrast, a limited number of studies have found reduced gene expression of ATGs in obese human adipocytes or adipose tissue. Obese adipose tissue expresses high levels of autophagy genes, but attenuated adipocyte autophagy flux. Weight loss by bariatric surgery can partly reverse flux attenuation, likely through the normalized expression of an autophagy regulator, DAPK2. In the present study, we did not find any transcriptional change in the expression of genes involved in the initiation complex or autolysosome stage. Therefore, our findings, together with those of others, suggest that overweight and obesity is accompanied by an upregulation of the autophagic flux in adipose tissue, predominantly due to an increase in the autophagic conjugation cascade.

The role of this increased adipose tissue autophagy is still unclear. It has been shown that ATG5 and ATG7 are required for differentiation of white adipocytes, increasing lipid storage and buffering capacity. Autophagy may be critical for the cytoplasmic remodeling necessary for trans-differentiation of brown AT to lipid-storing WAT such as through removal of the excessive mitochondria present in brown adipocytes. Alternatively, autophagy may promote the removal and degradation of a protein or proteins critical for the maintenance of the brown adipocyte phenotype. Rodents with AT-specific knock-out of ATG5 or ATG7 are characterized by a limited capacity of white AT to store triacylglycerol, suggesting that autophagy is essential for normal adipogenesis. ATG7 knock-out mice are much leaner than wild-type controls, and become resistant to high-fat-diet-induced obesity. Our present results show a negative correlation between BMI and ATGL and HSL, while a positive correlation between BMI and ATGs was observed. In fact, such a coordinated opposite regulation would fit well with a role in adipocyte differentiation, because differentiation of pre-adipocytes into mature adipocytes is paralleled by lipid storage and weight gain. According to the Gene Cards database (http://www.genecards.org) the promoter regions of the gene for ATG5, ATG7, ATG12 as well as ATGL share binding sites for several transcription factors like CTCF, ELF1 and GABPA in line with a coordinate regulation. No data were provided for HSL. However, resolving the exact regulatory mechanism requires further investigation. Taken together, these data suggest that autophagy might be involved in the adipose tissue expandability and lipid storage capacity, in particular via adipocyte differentiation.

In addition to a role in adipogenesis, our present findings indicate for the first time a potential role for autophagy in human adipose tissue lipid handling. Our cluster analysis showed a strong negative correlation between the expression of the autophagy pathway and classical lipolysis. This may indicate that impaired adipose tissue lipolysis, as often observed in the obese insulin resistant state, may activate autophagy-mediated lipid hydrolysis to deal with the increased lipid supply in obese AT. In line, it was recently shown that autophagy might contribute to lipid hydrolysis, at least in murine adipocytes. In line, our in vitro data of differentiated hMADS showed an increased LC3II/LC3I ratio following pharmacological HSL inhibition, indicating an increased starvation-induced autophagic flux in adipocytes. However, the exact mechanism and role of autophagy in adipocyte lipid metabolism remain to be investigated in more detail in future studies.

Obesity is associated with an elevated risk of developing insulin resistance and type 2 diabetes. Several studies have shown that insulin resistance and metabolic syndrome are distinctly influenced by autophagy in various tissues such as adipose tissue, skeletal muscles, pancreas, liver, and brain. In our study ATG7 also showed strong positive correlations with markers of glucometabolic status and insulin sensitivity including 2h-glucose, HOMA-IR and insulin. In line, adipose tissue-specific ATG7 knockout mice are lean and show markedly increased sensitivity and lower fasting plasma insulin level. Autophagic activity levels were increased in
adipose tissue of obese and insulin resistant animals compared to lean mice. Previous human studies showed that autophagy genes were more highly expressed in omental AT of obese insulin resistant than in age, sex, and BMI-matched insulin sensitive individuals, suggesting that autophagy is related to the degree of insulin resistance. Previous in vitro work has shown that insulin inhibits autophagic action in murine adipose tissue, and this may partly explain LC3 accumulation in adipose tissue of obese individuals characterized by insulin resistance. Regression analysis showed that the relationship between ATG and glucose tolerance is independent of insulin level. Moreover, our results show that 2 h glucose levels as a measure of glucose tolerance correlate negatively with ATGL and HSL mRNA levels in adipose tissue indicating that impaired glucose tolerance may be related to a decrease in classical lipolysis during the development of overweight/obesity. Since ATGL and HSL are also positively correlated with ATG7, we cannot exclude that the observed correlation between 2 h glucose and ATG7 is an indirect effect of its relation with classical lipolysis. More research is needed to clarify the cause and consequences of these interactions. It is well known that insulin inhibits autophagy through the activation of mTORC1. In line, insulin resistance will attenuate mTOR activity and consequently activate the autophagy pathway. In agreement with this, LC3II accumulation was found to correlate positively with HOMA-IR. In addition, the degree of insulin resistance was associated with elevated adipocyte autophagy flux in subcutaneous adipose tissue of obese diabetic patients. These data suggest that insulin resistance may underlie an increased expression of ATGs.

During the development of overweight and obesity, the hypertrophic adipocytes release chemokines and proinflammatory cytokines resulting in the activation of resident immune cells and the infiltration of additional immune cells into adipose tissue. While the adipocyte is the predominant cell type in healthy lean adipose tissue, immune cells can equal or even exceed fat cell number in obese adipose tissue. The resulting chronic low-grade inflammation of the adipose tissue is supposed to contribute to the development of impaired glucose tolerance and insulin resistance. Interestingly, there seems to be a relation between the inflammatory status of the adipose tissue and hyperactivity of the autophagy pathway. Consequently, elevated adipose tissue autophagy in obesity may restrain an “excessive” inflammatory response. Moreover, autophagy modulates the inflammatory status of adipose tissue by controlling the production of proinflammatory cytokines including IL-1β, IL-6 and IL-8. Inhibition of autophagy leads to an increase in gene expression and secretion of proinflammatory cytokines by the adipose tissue and by adipose tissue explants. Metabolically healthy obese, which is characterized by a lower inflammatory status of the adipose tissue, have a lower level of LC3-II in adipose tissue in line with a reduced need for an inflammatory dampening action by autophagy. Therefore, enhancement of autophagy in adipose tissue during obesity may serve to limit inflammation and prevent the development or further worsening of insulin resistance. Since we found that ATG7 correlates with impaired glucose tolerance in a BMI-independent way, autophagy-mediated regulation of adipose tissue inflammation may be an underlying factor linking autophagy to impaired glucose tolerance.

Finally, previous studies have shown that the increased expression of autophagy markers in obesity appears to be gender independent. In the current study, only 5 females were included which is too limited to make a valid conclusion whether the effects sex-dependent. Since gene expression is by far not the only level of regulating autophagy, a limitation of the current work is that we only measured mRNA expression. However, post-translational modification and subcellular localization, especially for the lipases, play important roles in determining lipolytic and autophagic fluxes, and therefore should be measured in adipocytes and tissues in future research when investigation potential cross-talk between both pathways. Finally, adipose tissue is composed of various cell types, of which the composition changes between lean and obese states. Therefore, we are not able at the moment to attribute our findings to a certain cell type. Of interest, recent data showed that in human adipocytes isolated from adipose tissue biopsies, the obese group showed higher p62 and lower LC3II indicating that the autophagic flux might rather be blunted in human obese adipocytes.

In conclusion, our results show that autophagy conjugation genes are up-regulated in subcutaneous adipose tissue of overweight/obese subjects. We further showed that the autophagy and classical lipolytic pathway are coordinately regulated in an opposite manner. Finally, we demonstrated that the ATG7 mRNA levels correlate with 2 h glucose indicating that autophagy in adipose tissue is linked to impaired glucose tolerance in a BMI- and insulin-independent way. Further investigation is needed to extend this knowledge and to shed more light on the metabolic involvement of autophagy in human adipocytes.

Materials and methods

Study population

Subcutaneous AT samples derived from two independent study cohorts were included in the present
analysis (18 from\textsuperscript{34}; 8 from\textsuperscript{35}). In total 9 lean 
(BMI $\leq$ 25 kg/m$^2$) glucose tolerant and 17 overweight/
obese individuals (BMI > 25 kg/m$^2$, $\leq$ 41.7 kg/m$^2$) with (n = 9) or without (n = 8) impaired glucose tolerance with-
out T2DM were included. Exclusion criteria were smoking, 
cardiovascular disease, cancer, lung disease, intentions to lose weight, weight change of more than 
3 kg within the 3 months before the study start, alcohol/drug abuse, use of antioxidants, and use of medication 
known to affect glucose metabolism or inflammation. The local Medical Ethical Committee of Maastricht Uni-
versity Medical center approved the study protocols and all participants gave a written informed consent in 
advance.

**Adipose tissue biopsy**

Abdominal subcutaneous AT biopsies ($\approx$1 g) were col-
lected 6 to 8 cm lateral from the umbilicus under local 
anesthesia (2% lidocaine) by needle biopsy after an over-
night fast. After immediate washing with saline, biopsy 
material was snap-frozen in liquid nitrogen and stored at 
$-80^\circ$C until analysis.

**Adipocyte size measurement**

All cells are stained by immunohistochemistry to allow 
identification of individual cells, and the inner cell 
radius/diameters of the circular cell cross-sections are 
determined from microscopy images by appropriate 
imaging software. In order to exclude cell fragments as 
well as cells from different cell types, or other small 
structures that are not of interest, the software is consid-
ered to have a lower cutoff, excluding everything that 
falls below this cutoff.\textsuperscript{36}

**In vivo phenotyping**

Anthropometric phenotyping was performed as de-
scribed before.\textsuperscript{34} Briefly, body weight was measured 
accurate to 0.1 kg on an electronic scale (Seca model 861, 
Hamburg, Germany) and height was measured accurate 
to 0.01 m. Hip circumference was measured over the 
greater trochanters directly over the underwear. Waist 
circumference was measured above the belly button and 
below the rib cage. WHR was the value of waist circumference divided by hip circumference. An oral glu-
cose tolerance test (OGTT) was performed after an over-
night fast to investigate glucometabolic status. Insulin 
sensitivity was assessed by calculating the HOMA-IR 
index using the formula described by Matthews et al.\textsuperscript{34}

**Gene expression and clustering**

Total RNA was extracted from tissue samples using 
TRIzol\textsuperscript{TM} Reagent (Ambion/Life 
Technologies; 15596–026). Reverse transcription of 
300 ng of total RNA was performed using the iScript 
cDNA synthesis kit (Bio-Rad; 170–8891). SYBR-Green 
base real-time PCRs were performed using an iCycler 
qPCR plates (Bio-Rad; 2239441; primer sequences see 
Supplementary Table S1). Reactions were performed in a 
total volume of 25 \mu l containing 5.5 \mu l cDNA, 12.5 \mu l 
iQ SYBR green supermix (BIO-RAD; 1708882) and 
gene-specific primers for NPRA and NPRC (Biolegio). Gene expression was normalized using 18S ribosomal 
RNA. Using RT-PCR we analyzed in the subcutaneous 
AT of the 26 participants the expression of several major 
autophagy-related genes involved in initiation (TFEB, 
RAB7A, RAB7B, ULK1, ULK2, BECN-1) and conjuga-
tion (ATG5, ATG12, ATG7, LC3A, LC3B), of the lys-
osomal lipase (LIPA), and of the major classical lipolysis-
related genes (ATGL, HSL).

**Cell culture experiments**

hMADS were cultured to 80% confluence and differenti-
ated for 7 days using differentiation medium (containing 
D-Pantothenate 17 \mu M, Insulin 0.1 \mu M, Dexamethasone 
1 \mu M, IBMX 250 \mu M, and Rosiglitazone 5 \mu M). There-
after, we removed the differentiation medium, then 
added another medium (containing D-Pantothenate 
17 \mu M, Insulin 0.1 \mu M) and refreshed the medium every 
2 days until day 13.\textsuperscript{37} Fully differentiated cells were 
washed twice with sterile PBS and incubated with or 
without an HSL inhibitor 25 \mu M (a kind gift from Chris-
tian Fledelius, Novo Nordisk, Copenhagen) in combina-
tion with the autophagy flux inhibitor Bafloymycin 
(Inivivogen; 88999–55-2) 100 nM to investigate links 
between classical lipolysis and autophagy. After 48 h 
incubation, the medium was removed and proteins were 
harvested by 1 x RIPA Lysis and Extraction Buffer 
(Thermo Scientific\textsuperscript{TM}; 89900) containing a protease 
inhibitor and phosphatase inhibitor cocktail (Cell Signal-
ning; 5872) for Western blot analysis.

**Western blot analysis**

For Western blot, 15 \mu g protein was subjected to 
SDS-PAGE and proteins were transferred to nitrocellu-
lose membrane (Criterion\textsuperscript{TM} Gel system). The mem-
brane for LC3 determine was blocked with 5% non-fat 
milk in PBST for 1 h at room temperature, followed by 
icubation overnight with primary antibodies: microtu-
bule-associated protein 1A/1B-light chain 3 (LC3) (Cell
Significance to mean values with their standard deviation (SD). Including all samples. Correlations with Pcalculated between the RNA levels of individual genes were two-tailed, and PChicago, IL, USA). All tests for statistical significance were performed with SPSS 23.0 for Windows (SPSS Inc., USA).

Differences between lean and overweight/obese groups were analyzed using an independent t-test. Relationships between clinical parameters and gene expression were tested by Pearson's correlation coefficients and multiple linear regression analyses. Backward stepwise elimination in multiple linear regressions was used to check whether combinations of characteristics might enhance the correlation with autophagy-related genes. Calculations were performed with SPSS 23.0 for Windows (SPSS Inc., Chicago, IL, USA). All tests for statistical significance were two-tailed, and P≤0.05 was considered statistically significant. Pearson's correlation coefficients were also calculated between the RNA levels of individual genes including all samples. Correlations with P≤0.01 were used to construct an interaction map. Data are presented as mean values with their standard deviation (SD).

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

Grants: This study was funded by an Innovative Pilot Research Grant (Grant No. 2008.11.010) from the Dutch Diabetes Research Foundation to Dr. Gijs H Goossens, an NWO-TOP Grant (Grant 200500001) to Pro. Edwin CM Mariman, and a fellowship for Qing Xu from China Scholarship Council (No.201407040041). Drugs: HSL inhibitor (a kind gift from Christian Fledelius, Novo Nordisk, Copenhagen)

Acknowledgments

We would like to thank all participants for participation in this study and N. Hoebers, Y. Essers for their excellent analytical and technical support.

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