Cathepsin gene expression in abdominal subcutaneous adipose tissue of obese/overweight humans

Qing Xu, Edwin C. M. Mariman, Gijs H. Goossens, Ellen E. Blaak, and Johan W. E. Jocken

Department of Human Biology, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Centre*, Maastricht, The Netherlands

ABSTRACT
Cathepsin L1 (CTSL1) and B (CTSB) are lysosomal proteases, of which the expression and activity are impaired in adipose tissue (AT) of obese rodents, indicating AT lysosomal dysfunction. Here we assess the relation between abdominal subcutaneous AT (SCAT) CTSL1 and CTSB gene expression (qRT-PCR), body composition and tissue-specific insulin resistance in 77 overweight/obese (BMI: 22.6–38.6 kg/m^2) well phenotyped men and women (61 M/16 F). A two-step hyperinsulinemic-euglycemic clamp was performed to assess AT, hepatic and skeletal muscle insulin sensitivity. Our data show that reduced CTSB expression is associated with markers of insulin resistance (standardized β = -0.561, p < 0.001), independent of adiposity, while CTSL1 expression is only associated with markers of body composition. Our data suggest the presence of lysosomal dysfunction in SCAT of obese humans with an impaired glucose homoeostasis. However, this needs to be investigated in more detail in future mechanistic studies.

Introduction
Impaired regulation of adipose tissue (AT) lipid metabolism may lead to excess lipid accumulation in ectopic tissues (e.g. skeletal muscle and liver), which contributes to the development of obesity-associated insulin resistance [1]. Of interest, impaired regulation of autophagy, a recycling system to maintain intracellular homoeostasis, has often been observed in AT of obese humans and rodents [2,3]. Recently, we showed that increased expression of autophagic genes (i.e. ATG 5–12 and 7) in human subcutaneous AT, was associated with an impaired glucometabolic status [4]. Lysosomal degradation of the autophagic cargo is the final stage of autophagy. Lysosomes contain over 50 functional enzymes (including glucosidases, proteases, sulfatases and others) for intracellular degradation of cellular components [5], which play an essential role in maintaining the autophagic clearance in adipose tissue [6]. Therefore, lysosomal dysfunction might play a key role in obesity-related metabolic disorders.

Cathepsins are a group of lysosomal proteases responsible for maintaining intracellular homoeostasis [7]. However, Cathepsin L1 (CTSL1) and Cathepsin B (CTSB) are the most abundant lysosomal proteases and participate directly in the execution of autophagy [8]. In addition, they have recently been implicated in lysosomal dysfunction and early pathologies of obese murine adipose tissue [7,9]. Recently, it was found that enhanced activation of CTSB protein and a concomitant decreased CTSL1 activation in AT is a marker of AT lysosome dysfunction in obese rodents, resulting in an attenuated lysosomal clearance and autophagosome accumulation [9]. Of interest, increased CTSB and CTSL1 mRNA expression was observed in the white adipose tissue of genetic (ob/ob) and high fat diet-induced obese mice, possibly contributing to obesity-associated adipose tissue dysfunction [9]. Therefore, CTSB and CTSL were of primary interest for our human explorative study in adipose tissue of obese metabolically compromised individuals. However, human evidence for lysosomal dysfunction within the obese adipose tissue is still scarce [9]. In this study, we performed a cross-sectional analysis in subcutaneous adipose tissue samples derived from 77 well phenotyped overweight/obese men and women of whom tissue-specific insulin sensitivity was determined using the gold standard hyperinsulinemic-euglycemic clamp with [6,6-2H_2]-glucose infusion [10]. CTSL1 and CTSB gene expression was measured using quantitative real time PCR (18 S was used as a housekeeping gene and data were calculated using delta CT method [11]) and related to detailed measures of glucose homoeostasis and adiposity.
Materials and methods

Study population

Subcutaneous AT (SCAT) samples were derived from two independent cohorts [12,13]. In total 77 low active (<3 h organized sports activities per week), weight-stable (<2 kg body weight change 3 months prior to inclusion) over-weight/obese Caucasian individuals (BMI 25–39 kg/m²) without type 2 diabetes were included. Cohort 1 consists of 50 men, age between 35 and 69 years with impaired fasting glucose (fasting glucose ≥6.1 mmol/L) and/or impaired glucose tolerance (2 h plasma glucose during a 75 g glucose tolerance test 7.8–11 mmol/L) and HOMA-IR>2.2. Cohort 2 consists of 27 subjects, men (n = 11) and women (n = 16) age between 21 and 50 years with normal glucose metabolism (fasting <6.1 mmol/L and 2 h <7.8 mmol/L). Our well-phenotyped cohorts included 19 overweight men and 8 overweight women, and 42 obese men and 8 obese women. Exclusion criteria were smoking, cardiovascular disease, cancer, lung disease, intentions to lose weight, alcohol/drug abuse, use of antioxidants, and use of medication known to affect glucose metabolism or inflammation. The local Medical Ethical Committee of Maastricht University Medical Centre approved the study protocols (ClinicalTrials.gov NCT02241421 and NCT02381145) and all participants gave a written informed consent in advance.

In vivo phenotyping and biochemical analysis

Anthropometric phenotyping was performed as described before [10,13]. 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 and waist circumferences were measured over the greater trochanters and above the belly button below the rib cage, respectively. Waist/hip ratio (WHR) was then calculated. Blood was collected into pre-chilled tubes and centrifuged at 1,000x g, and plasma was snap-frozen and stored at −80°C until analyses. Plasma glucose and FFA were determined using commercially available colorimetric assays on a Cobas Fara auto-analysers (Roche, Switzerland). Plasma insulin was measured with a double antibody radioimmunoassay (Millipore). Fasting insulin sensitivity was assessed by calculating the HOMA-IR index using the formula described by Matthews et al [14].

Hyperinsulinemic-euglycemic clamp

A two-step hyperinsulinemic-euglycemic clamp combined with a [6,6–3H2]-glucose tracer (Cambridge Isotope Laboratories) was performed to analyse the insulin-mediated suppression of free fatty acids (FFA suppression, representing AT insulin sensitivity), insulin-stimulated rate of disappearance of glucose (RdSS, representing peripheral/muscle insulin sensitivity), and insulin-mediated suppression of endogenous glucose production (%EGP suppression, representing hepatic insulin sensitivity). After a bolus-injection (2.4 mg/kg) [6,6–3H2]-glucose, tracer-infusion was started at 0.04 mg/kg/min, which was continued throughout the measurement. After 2 hours, low-dose insulin was infused at 10 mU/m²/min for 2 hours [15], followed by high-dose insulin at 40 mU/m²/min for 2 hours [16]. Blood samples were taken from a superficial dorsal hand vein, which was arterialized using a hot-box (50°C). By variable co-infusion of a 17.5% glucose solution, enriched by 1.1% tracer, plasma glucose concentrations were maintained at 5.0 mmol/L. For calculation of steady-state kinetics, three additional blood samples were taken every 10 min in the last 30 min of each step (0, 10, and 40 mU/m²/min insulin). The Rd was calculated during the 0- and 40-mU/m²/min insulin infusion, whereas calculations for insulin-mediated suppression of EGP and FFAs were performed during 0- and 10-mU/m²/min insulin infusion, as relative percentage of suppression during 10 compared with 0 mU/m²/min [10].

Adipose tissue biopsy

Abdominal subcutaneous AT (SCAT) biopsies were collected 6 to 8 cm lateral from the umbilicus under local anaesthesia (2% lidocaine) by needle biopsy after an overnight fast. After immediate washing with saline, biopsy material was snap-frozen in liquid nitrogen and stored at −80°C until mRNA analysis [13].

Adipose tissue mRNA analysis

Total RNA was extracted from tissue samples using TRizol® Reagent (Ambion/Life Technologies; 15,596–026). Reverse transcription of 300 ng of total RNA was performed using the iScript cDNA synthesis kit (Bio-Rad; 170–8891). SYBR-Green-based real-time PCRs were performed using an iCycler iQ Real Time PCR detection system (Bio-Rad). Reactions were performed in a total volume of 25 µl containing 5.5 µl cDNA, 12.5 µl iQ SYBR green supermix (Bio-RAD; 1,708,882) and gene-specific primers for CTSL1 (Biolegio, Forward Primer AAGTGAAGGCGATGGACAA, Reverse Primer AAAGCCATTCATCACCT GCC) and CTSL2 (Biolegio, Forward Primer TCGGA TGAGCTGGTCAACTA, Reverse Primer AGCTTCA GGTCCTCGGTTAAA). 18 S was used as a housekeeping
gene and data were calculated using delta CT method [17].

**Statistical analysis**

All variables were checked for normal distribution and variables with a skewed distribution were ln-transformed. Firstly, the Pearson’s correlation between CTSB and CTS1L mRNA expression were tested. Next, the associations of clinical parameters and gene expression were tested by Pearson’s correlation. Secondly, univariate regression was performed with CTSB or CTS1L gene expression as dependent variables and age, sex, BMI and WHR as independent variables (model 1). After that, to study the impact of CTSB or CTS1L on glucometabolic status and insulin resistance, univariate regression analysis was performed with fasting insulin, fasting glucose, HOMA-IR or HbA1 C as dependent variables, and RNA expression of CTSB or CTS1L as the independent factor adjusted by age, sex, BMI and WHR. Finally, to study the impact of CTSB or CTS1L on tissue-specific insulin sensitivity, univariate regression analysis was performed with FFA suppression, EGP and Rd entered separately as dependent variables, and RNA expression of CTSB or CTS1L as the independent factor adjusted by age, sex, BMI and WHR. 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.

**Results**

**Study population**

Clinical characteristics of the 77 overweight/obese participants are summarized in Table 1. Briefly, 16 females and 61 male overweight/obese participants were enrolled with an age range between 21 and 69 years, and broad range in BMI (25.6–38.6 kg/m²), fasting glucose (4.5–7.5 mM), fasting insulin (2.8–29.3 mU/L), and whole body insulin resistance (HOMA-IR 0.65–8.76) and tissue-specific insulin resistance (see Table 1).

**CTSB and CTS1L expression, body composition and tissue-specific insulin sensitivity**

A significant positive correlation was observed between CTSB and CTS1L mRNA expression in human SCAT (r = 0.419, p < 0.001). As shown in Table 2, CTSB mRNA expression did not correlate with markers of body composition (including BMI and WHR), but correlated negatively with age (r = −0.261, p = 0.024), fasting glucose (r = −0.299, p = 0.009), fasting insulin (r = −0.343, p = 0.003), HOMA-IR (r = −0.378, p = 0.001) and fasting FFA (r = −0.271, p = 0.036). The negative relationship between CTSB expression and fasting glucose (standardized β = −0.345, p = 0.034), insulin (standardized β = −0.505, p < 0.001), and HOMA-IR (standardized β = −0.561, p < 0.001) remained significant after adjustment for age, sex, BMI and WHR (Table 4). However, no associations were observed between AT, skeletal muscle and hepatic insulin sensitivity and SCAT CTSB expression (Table 5).

In contrast, CTS1L expression correlated positively with age (r = 0.352, p = 0.002), BMI (r = 0.363, p = 0.001), WHR (r = 0.521, p < 0.001), fasting glucose (r = 0.230, p = 0.046) and HbA1 C (r = 0.250, p = 0.031) (Table 2). Furthermore, CTS1L expression correlated negatively with insulin-induced FFA suppression (r = −0.372, p = 0.001), EGP suppression (r = −0.235, p = 0.049) and Rd (r = −0.341, p = 0.003) (Table 2). However, after adjustment age, sex, BMI, WHR, the associations between CTS1L SCAT expression and tissue-specific insulin sensitivity were no longer statistically significant (Tables 3–5).

**Table 1.** Participants’ characteristics.

|                | MEAN±SEM | RANGE         |
|----------------|----------|---------------|
| Male/female    | 61(42 obese)/16(8 obese) |               |
| Age (years)    | 52 ± 2   | 21–69         |
| Weight (kg)    | 94.8 ± 1.4 | 69.4–122.0    |
| Height (m)     | 1.75 ± 0.01 | 1.52–1.92    |
| BMI (kg/m²)    | 30.9 ± 0.3 | 25.6–38.6     |
| Waist (cm)     | 104 ± 1  | 77–126        |
| Hip (cm)       | 106 ± 1  | 89–125        |
| WHR            | 0.98 ± 0.01 | 0.70–1.22    |
| Fasting glucose (mM) | 13.9 ± 0.7 | 2.8–29.3     |
| Fasting insulin (mU/L) | 6.7 ± 0.2 | 3.4–11.2     |
| 2 h glucose (mM) | 3.6 ± 0.2 | 0.7–8.8      |
| HOMA-IR        | 5.4 ± 0.1 | 4.7–6.7      |
| HbA1 C (%)     | 615 ± 16 | 399–960      |
| FFA suppression (%) | 52.1 ± 2.3 | 6.1–90.1     |
| EGP suppression (%) | 48.5 ± 2.2 | 5.2–87.2     |
| Rd (μmol/kg/min) | 26.3 ± 1.2 | 9.8–54.0     |

Data are mean ± SD [Range: min-max]; BMI: body mass index; WHR: waist-hip ratio; HOMA-IR: homeostatic model assessment for insulin resistance, HbA1 C: haemoglobin A1 C, FFA: free fatty acids, EGP: endogenous glucose production, Rd: rate of disappearance.

**Table 2.** Pearson’s correlation coefficients for CTSB, CTS1L gene expression and clinical characteristics.

|                | Ln CTSB | Ln CTS1 |
|----------------|---------|---------|
| Age (years)    | −0.261* | 0.352** |
| BMI (kg/m²)    | 0.111   | 0.363** |
| WHR            | −0.076  | 0.521***|
| Fasting glucose (mM) | −0.343** | 0.178   |
| Fasting insulin (mU/L) | −0.435** | 0.187   |
| 2 h glucose (mM) | −0.047  | 0.221   |
| HOMA-IR        | −0.378**| 0.183   |
| HbA1 C (%)     | −0.033  | 0.250*  |
| Fasting FFA (μL) | −0.271* | 0.118   |
| FFA suppression (%) | −0.005  | −0.377**|
| EGP suppression (%) | 0.020   | −0.235* |
| Rd (μmol/kg/min) | 0.044   | −0.341**|

*p < 0.05, **p < 0.01, ***p < 0.001,
Table 3. Relationship between CTSB, CTSL1 gene expression in SCAT and adiposity.

| Dependent Variables (n = 77) | CTSL1 gene expression | CTSB gene expression |
|-----------------------------|-----------------------|----------------------|
|                             | standardized β [95%CI] | p value | Adj. R² | standardized β [95%CI] | p value | Adj. R² |
| BMI Model 1                  |                       |         |         |                       |         |         |
| BMI                          | 0.110 [−0.119, 0.339] | 0.345   | 0.012   | 0.369 [0.149, 0.588]  | 0.001** | 0.131   |
| Age                         | −0.430 [−0.814, −0.046] | 0.029*  | 0.009   | 0.196 [−0.039, 0.430] | 0.101   | 0.040   |
| Sex                         | 0.128 [−0.900, 1.156] | 0.805   | 0.001   | 0.404 [−0.499, 1.307] | 0.375   | 0.012   |
| Men                         |                       |         |         |                       |         |         |
| Women                       | 0.170 [−0.409, 0.750] | 0.560   | 0.005   | 0.378 [−0.131, 0.887] | 0.143   | 0.032   |

*All values entered in the model after z score standardization. *p < 0.05, **p < 0.01, ***p < 0.001

Discussion

In the present study, we examined the relationship between gene expression of the lysosomal proteases CTSB and CTSL1 in human SCAT, adiposity (i.e. BMI and WHR) and tissue-specific insulin sensitivity, in overweight/obese men and women. CTSB and CTSL1 mRNA expression in human SCAT were positively associated. Interestingly, a reduced SCAT CTSB mRNA expression was associated with increased whole-body insulin resistance (i.e. fasting glucose, insulin and HOMA-IR), independent of adiposity, while an increased SCAT CTSL1 mRNA expression was only associated with adiposity (i.e. BMI and WHR). However, both CTSB and CTSL1 expressions in the SCAT showed no significant association with tissue-specific insulin sensitivity following adjustment for age, sex, BMI, WHR. Together, our data indicate lysosomal dysfunction in the human adipose tissue of obese metabolically compromised humans.

The lysosomal proteases CTSB and CTSL1 are active as cysteine endo- and exopeptidase, which are abundantly expressed at the gene and protein level in human adipose tissue [8,18]. High expression of CTSB might contribute to increased basal lipolysis and a possible subsequent inflammatory response via reduce PLIN1 expression as shown in 3T3L-1 adipocytes [19]. Previously, observations in a relatively small study (n = 9) that showed CTSL1 gene expression is upregulated in SCAT of obese compared to lean 21–35 years-old men [20]. In contrast, the present data showed that CTSB expression in SCAT did not correlate with BMI or WHR in a large middle-aged population (age 52 ± 2 years).

It is well known that obesity is associated with insulin resistance of adipose tissue, the liver and skeletal muscle, reflected by whole-body insulin resistance [21]. However, the relation between SCAT lysosomal dysfunction and insulin resistance in obesity/overweight individuals has not been well studied. Autophagy is rapidly induced by nutrient deprivation (faster) and evidence is accumulating that this fasting-induced autophagy is defective regulated in insulin sensitive tissues such as liver, muscle, and adipose, in the context of obesity, which underpins an unprecedented role of autophagy in the manifestation of obesity-induced metabolic derangement [22,23]. Here we show that CTSB mRNA expression in SCAT is negatively correlated with fasting glucose and insulin levels, while no associations were observed between SCAT CTSB mRNA expression and tissue-specific insulin sensitivity, as determined by a 2-step hyperinsulinemic euglycemic clamp. In contrast, in rodent models it has been reported that whole-body pharmacological and genetic inactivation of CTSB protected against the development of hepatic steatosis and whole-body insulin resistance [24]. We previously observed in a sub-study of this cohort that plasma Cathepsin D (CTSD) activity, but not systemic inflammation, is inversely related to hepatic insulin sensitivity, suggesting that plasma CTSD activity may be used as a non-invasive marker for hepatic insulin sensitivity in humans [25]. The observed fasting lysosomal dysfunction might compromise the ability of the cell to perform quality control on the mitochondrial matrix, since autophagy plays a pivotal role in the degradation of defective mitochondria. Similarly, autophagy also plays an indispensable role in the clearance of protein aggregates and redundant large protein platforms such as inflammasomes. Furthermore, autophagy might also play a key role in the metabolism of endotoxins, implicating the importance of autophagy in the pathogenesis of metabolic endotoxemia [26]. Together, these data argue for species-specificity concerning the action of cathepsins on the development of whole-body insulin resistance, which is important to recognize and warrants further investigation.

Finally, we observed that in abdominal SCAT of overweight/obese men and women, expression of CTSL1 was positively associated with adiposity). This finding is in line with a previous study in diet-induced and genetically obese mice (ob/ob mice), which were
characterized by lower CTSL1 protein expression and activity in white AT (WAT) compared to lean control animals, while the mRNA expression of CTSL1 and pro-CTSL1 was increased in WAT of obese mice [9]. These data point towards an impaired lysosomal function in human obesity. However, this needs to be investigated in more detail in future research using functional measurements of SCAT lysosomal activity.

Of interest, we observed a strong positive correlation between CTSL1 and CTBS expression in human SCAT. However, pharmacological inhibition or genetic knockdown of CTSL1 induced a compensatory transcriptional upregulation and enzymatic activation of CTBS in murine 3T3 L1 adipocytes, which was accompanied by increased autophagosome accumulation, possibly reflecting lysosomal dysfunction [9]. In contrast to CTSL1, CTBS protein expression and its enzymatic activity were increased in WAT of HFD-induced obese mice [9]. This enhanced activation of CTBS promotes CTSL cleavage, resulting in further suppression of CTSL enzymatic activity, which leads to impaired autophagic clearance and autophagosome accumulation in WAT of obese mice [18]. Therefore, it will be of importance to establish whether the observed transcriptional changes in human SCAT also translate into changes at the protein level and are accompanied by changes in CTSL1 and CTBS activity in obese/overweight men and women.

In future studies, it would be of interest to also investigate other cathepsin families in relation to human adipose tissue lysosomal dysfunction. In addition, it would be interesting to investigate cathepsin tissue expression, activity and plasma levels in lean as compared to overweight/obese humans, and include morbidly obese (BMI>35 kg/m²) individuals to investigate the effects of body weight and body composition per se. Moreover, future studies should also investigate the effect of lysosomal dysfunction on human adipocyte function and substrate metabolism using in vitro mechanistic approaches and to explore the road to modulate human adipose tissue lysosomal function via nutritional, pharmacological and lifestyle interventions aimed at improving metabolic health.

In summary, CTSL1 expression in human SCAT is positively associated with adiposity, while SCAT CTBS expression is inversely related to whole-body insulin resistance. Together, these data suggest that lysosomal dysfunction, reflected by increased CTSL1 and decreased CTBS expression, is present in SCAT of overweight/obese men and women, and may relate to impaired whole-body glucose homoeostasis. Nevertheless, our data are correlational in nature and further research is required to determine the causality of these associations.
Table 5. Relationship between CTSB, CTSL1 gene expression in SCAT and tissue-specific insulin sensitivity.

| Dependent Variables | FFA suppression | EGP suppression | RdSS |
|---------------------|-----------------|-----------------|------|
|                     | standardized β [95% CI] | p value | Adj R² | standardized β [95% CI] | p value | Adj R² | standardized β [95% CI] | p value | Adj R² |
| CTSB gene expression | 0.029[-0.166,0.225] | 0.765 | 0.001 | 0.054[-0.187,0.295] | 0.656 | 0.003 | -0.013[-0.213,0.188] | 0.901 | 0.000 |
| CTSL1 gene expression | -0.018[-0.227,0.191] | 0.864 | 0.000 | 0.066[-0.198,0.331] | 0.617 | 0.004 | 0.000[-0.226,0.226] | 0.999 | 0.000 |

All values entered in the model after z score standardization and adjusted for age, sex, BMI, WHR.

Acknowledgments

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

Disclosure statement

All authors contributed significantly to the study and approved the manuscript for submission. None of the authors declare a conflict of interest in relation to this manuscript.

Funding

This study was partly funded by TI Food and Nutrition a public-private partnership on pre-competitive research on food and nutrition [grant to EEB and GHG], the alpro Foundation [grant to EEB], and a fellowship for QX from the China Scholarship Council [No.201407040041].

ORCID

Edwin C. M. Mariman @ http://orcid.org/0000-0002-5691-8633

References

[1] Blühler M. Adipose tissue dysfunction in obesity. Exp Clin Endocrinol Diabetes. 2009;117(6):241–250.
[2] Nunez C, Rodrigues VS, Gomes FS, et al. Defective regulation of adipose tissue autophagy in obesity. Int J Obesity. 2013;37(11):1473.
[3] Soussi H, Reggio S, R Alili, et al. DAPK2 down-regulation associates with attenuated adipocyte autophagic clearance in human obesity. Diabetes. 2015;64(10):3452–3463.
[4] Xu Q, Mariman ECM, Roumans NJT, et al. Adipose tissue autophagy related gene expression is associated with glucometabolic status in human obesity. Adipocyte. 2018;7(1):12–19.
[5] Fujiwara Y, Wada K, Kabuta T. Lysosomal degradation of intracellular nucleic acids—multiple autophagic pathways. J Biochem. 2016;161(2):145–154.
[6] Meijer AJ, Codogno P. Regulation and role of autophagy in mammalian cells. Int J Biochem Cell Biol. 2004;36(12):2445–2462.
[7] Araujo TF, Cordeiro AV, Vasconcelos DAA, et al. The role of cathepsin B in autophagy during obesity: a systematic review. Life Sci. 2018;209:274–281.
[8] Kaminsky V, Zhivotovsky B. Proteases in autophagy. Biochim Biophys Acta. 2012;1824(1):44–50.
[9] Mizunoe Y, Sudo Y, Okita N, et al. Involvement of lysosomal dysfunction in autophagosome accumulation and early pathologies in adipose tissue of obese mice. Autophagy. 2017;13(4):642–653.
[10] Most J, Timmers S, Warnke I, et al. Combined epigallocatechin-3-gallate and resveratrol supplementation for 12 wk increases mitochondrial capacity and fat oxidation, but not insulin sensitivity, in obese humans: a randomized controlled trial. 2. Am J Clin Nutr. 2016;104(1):215–227.
[11] Rao X, Huang X, Zhou Z, et al. An improvement of the Z’ (–delta delta CT) method for quantitative real-time polymerase chain reaction data analysis. Biostat Bioinforma Biomath. 2013;3(3):71.
[12] Most J, Goossens GH, Reijnders D, et al. Gut microbiota composition strongly correlates to peripheral insulin sensitivity in obese men but not in women. Benef Microbes. 2017;8(4):557–562.
[13] Reijnders D, Goossens G, Hermes GA, et al. Effects of gut microbiota manipulation by antibiotics on host metabolism in obese humans: a randomized double-blind placebo-controlled trial. Cell Metab. 2016;24(1):63–74.
[14] Matthews D, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412–419.
[15] Kotronen A, Seppälä-Lindroos A, Bergholm R, et al. Tissue specificity of insulin resistance in humans: fat in the liver rather than muscle is associated with features of the metabolic syndrome. Diabetologia. 2008;51(1):130–138.
[16] Brehm A, Krssak M, Schmid AI, et al. Increased lipid availability impairs insulin-stimulated ATP synthesis in human skeletal muscle. Diabetes. 2006;55(1):136–140.
[17] Jocken JW, Langin D, Smit E, et al. Adipose triglyceride lipase and hormone-sensitive lipase protein expression is decreased in the obese insulin-resistant state. J Clin Endocrinol Metab. 2007;92(6):2292–2299.
[18] Turk V, Stoka V, Vasiljeva O, et al. Cysteine cathepsins: from structure, function and regulation to new frontiers. Biochim Biophys Acta. 2012;1824(1):68–88.
[19] Mizunoe Y, Kobayashi M, Hoshino S, et al. Cathepsin B overexpression induces degradation of perilipin 1 to cause lipid metabolism dysfunction in adipocytes. Sci Rep. 2020;10(1):1–12.
[20] Gonzalez-Muniesa P, Marrades M, Martinez J, et al. Differential proinflammatory and oxidative stress response and vulnerability to metabolic syndrome in habitual high-fat young male consumers putatively...
predisposed by their genetic background. Int J Mol Sci. 2013;14(9):17238–17255.

[21] Kahn BB, Flier JS. Obesity and insulin resistance. J Clin Invest. 2000;106(4):473–481.

[22] Bagherniya M, Butler AE, Barreto GE, et al. The effect of fasting or calorie restriction on autophagy induction: A review of the literature. Ageing Res Rev. 2018;47:183–197.

[23] Kim KH, Jeong YT, Oh H, et al. Autophagy deficiency leads to protection from obesity and insulin resistance by inducing Fgf21 as a mitokine. Nat Med. 2013;19(1):83.

[24] Feldstein AE, Werneburg NW, Canbay A, et al. Free fatty acids promote hepatic lipotoxicity by stimulating TNF-α expression via a lysosomal pathway. Hepatology. 2004;40(1):185–194.

[25] Ding L, Goossens GH, Oligschlaeger Y, et al. Plasma cathepsin D activity is negatively associated with hepatic insulin sensitivity in overweight and obese humans. Diabetologia. 2020;63(2):374–384.

[26] van Niekerk G, Du Toit A, Loos B, et al. Nutrient excess and autophagic deficiency: explaining metabolic diseases in obesity. Metabolism. 2018;82:14–21.