Communication

Branched-Chain Fatty Acids Alter the Expression of Genes Responsible for Lipid Synthesis and Inflammation in Human Adipose Cells

Aleksandra Czumaj *, Tomasz Śledziński and Adriana Mika

Department of Pharmaceutical Biochemistry, Faculty of Pharmacy, Medical University of Gdańsk, Debinki, 80-211 Gdańsk, Poland; tomasz.sledzinski@gumed.edu.pl (T.Ś.); adriana.mika@gumed.edu.pl (A.M.)
* Correspondence: aczumaj@gumed.edu.pl; Tel.: +48-58-523-51-90

Abstract: Recently, we have demonstrated a decreased level of iso-branched-chain fatty acids (iso-BCFAs) in patients with excessive weight. However, it is still unclear whether BCFAs may influence lipid metabolism and inflammation in lipogenic tissues. To verify this, human visceral adipocytes were cultured with three different concentrations of selected iso-BCFA (14-methylpentadecanoic acid) and anteiso-BCFA (12-methyltetradecanoic acid), and then the expression of genes associated with lipid metabolism (FASN—fatty acid synthase; SREBP1—sterol regulatory element-binding protein 1; SCD1—stearoyl-CoA desaturase; ELOVL4—fatty acid elongase 4; ELOVL6—fatty acid elongase 6; FADS2—fatty acid desaturase 2; FADS1—fatty acid desaturase 1) and inflammation (COX-2—cyclooxygenase 2; ALOX-15—lipoxygenase 15; IL-6—interleukin 6) were determined. This study demonstrates for the first time that incubation with iso-BCFA decreases the expression of adipocyte genes that are associated with lipid metabolism (except FASN) and inflammation. These findings suggest that changes in the iso-BCFA profile in obese patients may contribute to adipose inflammation and dyslipidemia. Further studies should evaluate whether iso-BCFA supplementation in obese patients would be beneficial.

Keywords: iso-BCFA; anteiso-BCFA; branched-chain fatty acids; adipocytes; obesity; inflammation

1. Introduction

Obesity is a complex chronic disease that adversely affects nearly all physiological functions of the body. It contributes to reduced life expectancy, impaired quality of life, and increases the risk of developing multiple disease conditions, such as type 2 diabetes (T2D); non-alcoholic fatty liver disease (NAFLD); hypertension; coronary heart disease; stroke; and several types of cancer [1–4]. The well-known hallmark of obesity is dyslipidemia [5,6]. One group of fatty acids that is gaining research interest in this matter is branched-chain fatty acids (BCFAs). BCFAs are a class of mostly saturated fatty acids with one or more methyl branches in their carbon chains. Based on branch point position, the following types are distinguished: iso-BCFA (with a methyl branch on the penultimate carbon, i.e., one from the end) and anteiso-BCFA (with the methyl branch located on the antepenultimate carbon, i.e., two from the end), (Table 1) [7,8]. Until now, in humans, BCFAs have been found in vermix caseosa, breast milk, adipose tissue, and serum [9–12]. Moreover, there is growing evidence that BCFAs are associated with obesity and inflammation. In our previous studies, we showed that in the serum of obese patients the levels of BCFAs, especially iso-BCFA, were lower in comparison to non-obese patients [12,13]. Other authors also reported similar results but in adipose tissue [9–12]. Moreover, there is growing evidence that BCFAs are associated with obesity and inflammation. In our previous studies, we showed that in the serum of obese patients the levels of BCFAs, especially iso-BCFA, were lower in comparison to non-obese patients [12,13]. Other authors also reported similar results but in adipose tissue [9–12]. However, the consequences of these changes are still not explored. Moreover, the anti-inflammatory properties of BCFA were mostly studied in the context of dietary BCFAs and gastrointestinal health [14–16]. In the present study we focus on adipocyte inflammation since several researchers have confirmed that low-grade inflammation of adipose tissue is associated with obesity-related metabolic diseases [17–20].
We also found statistically significant inverse correlations between the serum concentration of iso-BCFA and triglycerides (TG), as well as C-reactive protein (marker of inflammation) in obese patients [12]. However, in our previous research, we only speculated about a possible molecular mechanism of this relationships. In this paper, we investigated whether changes in BCFA level are just another disorder associated with dyslipidemia and inflammation observed in obese patients or, if these BCFA alterations may play a role in the development of dyslipidemia and inflammation by affecting the adipocytes, one of the main types of cells involved in lipid metabolism and inflammation in humans.

The aim of this study was to analyze the effect of selected BCFAs on the expression of genes related to lipid synthesis and inflammation in adipocytes.

2. Materials and Methods
2.1. Cell Culture and Treatment

We used primary human white preadipocytes that were isolated from adult visceral adipose tissue. The cells, all media and supplements were purchased from PromoCell (PromoCell GmbH, Heidelberg, Germany). The cells were cultured and differentiated according to the manufacturer’s instructions. In brief, the preadipocytes were plated with a plating density of 5000 cells per cm² on 6-well plates and cultured in preadipocyte basal medium supplemented with fetal calf serum (final concentration: 0.05 mL/mL); endothelial cell growth supplement (0.004 mL/mL); recombinant human epidermal growth factor (10 ng/mL); hydrocortisone (1 μg/mL); and heparin (90 μg/mL). After the cells reached total confluency stage, preadipocyte growth medium was replaced by preadipocyte differentiation medium for 72 hours. Preadipocyte differentiation medium was prepared from basal medium; d-Biotin (8 μg/mL); recombinant human insulin (0.5 μg/mL); dexamethasone (400 ng/mL); IBMX (44 μg/mL); L-thyroxine (9 ng/mL); and ciglitazone (3 μg/mL). After 72 h, preadipocyte differentiation medium was replaced by adipocyte nutrition medium (preadipocytes basal medium supplemented with fetal calf serum (0.03 mL/mL); d-Biotin 8 (μg/mL); recombinant human insulin (0.5 μg/mL); and dexamethasone (400 ng/mL). The medium was changed every 2 days. After 2 weeks the differentiation process was complete and only mature adipocytes were present on the plates. Adipocytes differentiation was confirmed by oil red O staining. All cells were cultured in a humidified atmosphere of 95% air and 5% CO₂ at 37 °C.

The mature visceral adipocytes were incubated with 14-methylpentadecanoic acid (iso-palmitic acid, iso 16:0, 14-MPA) or 12-methyltetradecanoic (anteiso-pentadecanoic acid, anteiso 15:0, 12-MTA). These specific BCFAs were selected based on our previous research.

Table 1. Structural differences among straight-chain fatty acid, iso- and anteiso-BCFA. BCFA—branched-chain fatty acid.

| Type of Fatty Acid | 15 Carbon-Atom Fatty Acid |
|-------------------|---------------------------|
| Straight-chain fatty acid | Pentadecanoic acid |
| Iso-BCFA          | 13-Methyltetradecanoic acid |
| Anteiso-BCFA      | 12-Methyltetradecanoic acid |

We also investigated whether changes in BCFA level are just another disorder associated with dyslipidemia and inflammation observed in obese patients or, if these BCFA alterations may play a role in the development of dyslipidemia and inflammation by affecting the adipocytes, one of the main types of cells involved in lipid metabolism and inflammation in humans.

The aim of this study was to analyze the effect of selected BCFAs on the expression of genes related to lipid synthesis and inflammation in adipocytes.
The 14-MPA was selected because it was a branched-chain fatty acid whose serum content significantly statistically differed between patients with excess weight and healthy subjects with normal weight. The 12-MTA was selected based on similar serum levels in obese patients and a similar length of the carbon chain to the 14-MPA. Due to the similar serum contents of both BCFAs, we were able to use the same experimental concentrations. The abovementioned BCFAs were used in three different concentrations: 2 µM, 5 µM, and 10 µM for 48 h. The concentrations were selected to mimic normal physiological conditions (5 µM) and states of decreased and increased BCFA concentrations, 2 µM and 10 µM, respectively. BCFAs were purchased from Sigma-Aldrich (St. Louis, MO, USA). Adipocytes in basal adipocyte nutrition medium were used as a control. The selected concentrations of BCFAs did not influence the cells’ viability when assessed by MTT assay. Due to the limited possible number of passages of primary human white preadipocytes, the number of experiments that can be performed with these cells is limited. We used whole material that was obtained from purchased cells to perform the experiments presented in this paper.

2.2. Real-Time PCR Analysis of mRNA Levels

Cells were lysed directly on the culture plate with the QIAzol Lysis Reagent (Qiagen) after medium removing and PBS washing. The total RNA was isolated from the cells with an RNeasy Lipid Tissue Mini Kit (Qiagen, Hilden, Germany). RNA quantity and purity were determined by optical density and A260/280 and A260/230 ratio using a NanoDrop One Microvolume UV-Vis Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The integrity of RNA was assessed by RNA StdSens Assay on an Experion Automated Electrophoresis System (Bio-Rad Laboratories, Hercules, CA, USA). An amount of 1 µg of total RNA was reverse transcribed using a RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA, USA)). Real-time PCR was performed on a CFX Connect Real-Time PCR Detection System (Bio-Rad) using a SensiFAST SYBR No-ROX Kit (Meridian Bioscience, Cincinnati, OH, USA). All primers were synthesized by Genomed S.A. (Warsaw, Poland). The specificity of the mRNA amplification was confirmed by melting curve analysis. Real-time PCR data were analyzed using the $2^{-\triangle\triangle\Delta Ct}$ relative quantification method.

2.3. Statistical Analysis

All data are presented as mean ± SD. All experimental conditions were analyzed in triplicates in three independent experiments. The statistical significance of the differences between the experimental and control conditions was verified with the Mann–Whitney U test. The results were considered significant for $p$-values < 0.05. All analyses were conducted using Statistica 13 (StatSoft, Cracow, Poland).

3. Results and Discussion

Incubation with 12-MTA increased the expression of adipocytes genes encoding enzymes involved in fatty acid synthesis (FASN); elongation of polyunsaturated fatty acids (PUFA)–ELOVL4; and elongation of saturated (SFA) and monounsaturated fatty acids (MUFA)–ELOVL6. In contrast, the relative expression level of gene encoding stearoyl-CoA desaturase (SCD1) was decreased. The 12-MTA did not change the expression level of genes encoding fatty acid desaturases involved in PUFA metabolism (FADS1, FADS2), (Figure 1).
Incubation with 14-MPA increased only the expression of gene-encoding FASN. In contrast to 12-MTA, 14-MPA had the opposite effect on the expression level of genes encoding ELOV4 and ELOV6. The decreased expression level was observed for genes encoding sterol regulatory element-binding protein 1 (SREBP1c) and FADS1 (Figure 2). Similar to 12-MTA, 14-MPA did not change the expression of FADS2.

In vivo and in vitro studies have shown that in adipose tissue SCD1 is involved in the promotion of lipid mobilization by enhancing lipolysis and lipogenesis [21,22]. SCD1 is a key enzyme involved in de novo lipogenesis and is responsible for the conversion of SFAs to MUFAs. MUFAs such as, for example, palmitoleate (16:1) and oleate (18:1) can then be used for TG synthesis. Thus, changes in SCD1 activity/expression are related to TG level [23]. Furthermore, studies have shown that oleic acid, a product of SCD1, may upregulate the expression of adipose TG lipase (ATGL) and hormone-sensitive lipase (HSL) in adipose tissue [24–26]. Therefore, the increased expression of SCD1 may lead to enhancing the release of free fatty acids (FFAs) from adipose tissue. These FFAs can be re-esterified in adipose tissue to form TG. Moreover, in obese subjects, a higher mRNA level
of SCD1 was observed in comparison to non-obese subjects [27]. Therefore, an increased production and release of FFAs from adipose tissue can contribute to dyslipidemia that is observed in obese subjects. We show in this study that both iso- and anteiso-BCFAs are able to decrease the expression level of SCD1; however, iso-BCFAs are decreased in obese subjects [12]. SREBP1c is a well-known transcription factor that activates genes involved in FA and TG synthesis, including SCD1 [28,29]. Decrease in SREBP1c mRNA level suggest a possible molecular mechanism by which iso-BFCAs can influence SCD1 gene expression and lipid metabolism.

We observed that under the influence of BCFA, the mRNA levels of SCD1 and FASN changed in the opposite way. Although FASN can be also regulated by SREBP1 [30], our results suggest that in adipocytes, FASN expression after BCFA treatment may be regulated in an SREBP1-independent manner [31]. Opposite changes in the expression level of these lipogenesis genes (SCD1 and FASN) were also observed by Eissing et al. [27] who observed that in the visceral white adipose tissue of obese subjects, mRNA levels of FASN were lower and SCD1 mRNA were higher in comparison to non-obese subjects [27].

Studies have shown that ELOVL6 has a crucial role in the development of obesity-induced pathologies. Mice with ELOVL6 deficiency were protected from hyperinsulinemia, hyperglycemia, and hyperleptinemia [32–34]. In this study, we demonstrated that iso-BCFA has the ability to decrease the expression level of ELOVL6. Since the concentration of this type of BCFAs is lower in the serum of obese subjects, it can be speculated that supplementation of iso-BCFAs may lead to the improvement of insulin sensitivity in obese subjects.

BCFAs can also alter the expression of the genes that are involved in inflammation (Figure 3a,b). The greatest effect was observed for interleukin 6 (IL-6). Interestingly, iso-BCFA and anteiso-BCFA had the opposite effect on the expression of this gene. The 14-MPA, a representative of iso-BCFA, decreased the expression of IL-6 in the dose-dependent matter (Figure 3b). However, this type of BCFA is decreased in subjects with obesity. IL-6 is a major inflammatory mediator involved in obesity-related chronic inflammation, which may result in an increased risk of cardiovascular complication, insulin resistance, and type 2 diabetes [35,36]. Until now, several physiological or pathological factors were connected with the IL-6 level, including hormones, diet, exercise, and stress [37–40]. This study demonstrates for the first time that BCFA may also influence the expression level of IL-6 in adipocytes. It has been shown that IL-6 can promote the synthesis of C-reactive protein (CRP) [41,42]. Therefore, the level of iso-BCFAs may indirectly influence the level of CRP. This may explain why in our previous work we document the inverse correlation between serum CRP and iso-BCFA levels [12].

![Figure 3](image_url)

**Figure 3.** Relative expression level of selected genes involved in inflammation in visceral adipocytes incubated for 48 h in different concentrations of (a) 12-methyltetradecanoic (12-MTA) and (b) 14-methylpentadecanoic acid (14-MPA). Data are presented as mean ± SD. * p < 0.05 compared to control. COX-2—cyclooxygenase 2; ALOX-15—lipoxgenase 15; IL-6—interleukin 6.
Incubation of visceral adipocytes with both types of BCFA (iso-, and anteiso-BCFA) resulted in the decreased expression level of ALOX-15 (Figure 3a,b). The expression of various lipoxygenases (LOX) isoforms, including ALOX-15, were reported in human visceral adipose tissue [43,44]. LOX also plays an important role in obesity and obesity-induced consequences, such as inflammation and insulin resistance [45–49]. Enzymes that are encoded by ALOX-15 generate various bioactive lipid mediators, such as eicosanoids, hepoxilins, lipoxins, and other molecules from various PUFA substrates [48,50,51]. For example, 15-lipoxygenase converts arachidonic acid (AA) into 15-hydroxyeicosatetraenoic acid (15(S)-HETE), a known pro-inflammatory molecule [52–54]. Studies have shown that the level and activity of LOX-15 are increased in mice on a high-fat diet and obese patients [35]. This study has shown for the first time that a reduced level of BCFA in obese subjects can be one of the possible molecular mechanisms of this phenomenon.

This study has shown that 12-MTA, an anteiso-BCFA, did not affect the expression level of cyclooxygenase 2 gene (COX-2), while 14-MPA caused a statistically significant reduction in expression, only at the highest experimental concentration (Figure 3a,b). COX-2 generates pro-inflammatory mediators—prostaglandins (mainly PGE2) from AA. Studies have shown that the expression level of COX-2 is elevated in the subcutaneous adipose tissue of obese humans, and that COX-2 is involved in the development of obesity-associated adipose tissue inflammation and insulin resistance [55–57]. This study demonstrates for the first time that BCFA can modulate COX-2 expression.

Based on the results presented in this study, it can be concluded that iso-BCFA can be another regulatory factor involved in regulating inflammation, mainly by decreasing the expression level of pro-inflammatory genes such as COX-2, IL-6 and ALOX-15. Lower iso-BCFA levels observed in obese patients can aggravate inflammation in adipose tissue and increase the risk of obesity-related metabolic diseases. This supposition is supported by the fact that an increase in iso-BCFA levels in obese subjects that lost body weight after bariatric surgery was associated with decreased inflammation (assessed based on serum CRP levels) [13]. It can be speculated that iso-BCFA may find potential applications as protective agents against obesity-induced consequences. Since the main source of BCFAs for humans is the dietary intake of common dairy products, modifying the diet of obese patients for BCFA content could be beneficial in terms of hyperlipidemia and inflammation reduction. However, to date, human trials assessing the health effects of the supplementation of iso-BCFA are still lacking and animal data are very limited. Moreover, there are currently no recommendations for the daily intake of BCFA. Very little is known about the daily intake of BCFAs in humans. Based on the daily intake of dairy and beef products in the United States, the daily intake of BCFA has been estimated to range from 220 mg/day to 500 mg/day [58,59]. To the authors’ knowledge there are no such studies from other countries. Moreover, diet is not the only source of BCFA in humans. Some studies have shown that BCFAs can be produced by gut microbiota [60] or can by synthesized de novo in mammals [11,61]. Therefore, further investigations are required.

In the present study, the effects of BCFA were evaluated in visceral adipocytes because this is the main adipose tissue that is associated with obesity-related diseases [62,63]. Taking into consideration that lipid metabolism can be very depot-dependent, to better understand the comprehensive effect of BCFA on lipid metabolism, adipocytes from other adipose tissue should be analyzed in further research.

The limitation of this study is the fact that the expression of genes was studied only on the mRNA levels and not on the protein levels. The commercially available primary pre-adipocytes that were purchased for our experiments can be cultured for a limited number of passages, and the number of cells that were obtained after the differentiation of adipocytes allowed only for mRNA experiments. However, there is strong evidence that the expression of studied genes is regulated on the level of transcription [21,64,65].
4. Conclusions

In conclusion, this study demonstrates for the first time that iso-BCFAs can decrease the expression level of genes that are involved in lipid synthesis (except for FASN) and genes that encode pro-inflammatory proteins in a dose-dependent manner. Based on the results presented in this study, one can suppose that the decreased level of iso-BCFA that was previously observed in obese subjects may contribute to dyslipidemia and inflammation. Further studies should evaluate whether iso-BCFA supplementation in obese patients would be beneficial.

Author Contributions: Conceptualization, A.C., T.S. and A.M.; methodology, A.C.; investigation, A.C. writing—original draft preparation, A.C.; writing—review and editing, A.C., T.S. and A.M.; visualization, A.C.; supervision, T.S.; funding acquisition, A.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Science Centre of Poland, grant number NCN 2016/21/D/NZ5/00219, and by the Medical University of Gdansk (grants no. ST-40).

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Hall, J.E.; do Carmo, J.M.; da Silva, A.A.; Wang, Z.; Hall, M.E. Obesity-Induced Hypertension. *Circ. Res.* 2015, 116, 991–1006. [CrossRef] [PubMed]

2. Longo, M.; Zatterale, F.; Naderi, J.; Parrillo, L.; Formisano, P.; Raciti, G.A.; Beguinot, F.; Miele, C. Adipose Tissue Dysfunction as Determinant of Obesity-Associated Metabolic Complications. *Int. J. Mol. Sci.* 2019, 20, 2358. [CrossRef] [PubMed]

3. Chooi, Y.C.; Ding, C.; Magkos, F. The epidemiology of obesity. *Metabolism* 2019, 92, 6–10. [CrossRef] [PubMed]

4. Van Gaal, L.F.; Mertens, I.L.; De Block, C.E. Mechanisms linking obesity with cardiovascular disease. *Nature* 2006, 444, 875–880. [CrossRef] [PubMed]

5. Zhang, T.; Chen, J.; Tang, X.; Luo, Q.; Xu, D.; Yu, B. Interaction between adipocytes and high-density lipoprotein: new insights into the mechanism of obesity-induced dyslipidemia and atherosclerosis. *Lipids Health Dis.* 2019, 18, 223. [CrossRef] [PubMed]

6. Blüher, M. Metabolically Healthy Obesity. *Endocr. Rev.* 2020, 41, 405–420. [CrossRef]

7. Ran-Ressler, R.R.; Glahn, R.P.; Brenna, J.T. Branched Chain Fatty Acids (BCFA) in the Neonatal Gut, and Estimated Dietary Intake in Infancy and Adulthood; Nestle Nutrition Institute Workshop Series; Nestec Ltd.: Basel, Switzerland, 2013; Volume 77, p. 133. [CrossRef] [PubMed]

8. Taormina, V.M.; Unger, A.L.; Schiksnis, M.R.; Torres-Gonzalez, M.; Kraft, J. Branched-Chain Fatty Acids—An Undersampled Class of Dairy-Derived Fatty Acids. *Nutrients* 2020, 12, 2875. [CrossRef]

9. Ran-Ressler, R.R.; Devapatla, S.; Lawrence, P.; Brenna, J.T. Branched Chain Fatty Acids Are Constituents of the Normal Healthy Newborn Gastrointestinal Tract. 2008. Available online: www.lipidlibrary.co.uk (accessed on 22 May 2022).

10. Dingess, K.A.; Valentine, C.J.; Ollberding, N.J.; Davidson, B.S.; Woo, J.; Summer, S.; Peng, Y.M.; Guerrero, M.L.; Ruiz-Palacios, G.M.; Ran-Ressler, R.R.; et al. Branched-chain fatty acid composition of human milk and the impact of maternal diet: The Global Exploration of Human Milk (GEHM) Study. *Am. J. Clin. Nutr.* 2017, 105, 177–184. [CrossRef]

11. Su, X.; Magkos, F.; Zhou, D.; Eagon, J.C.; Fabbrini, E.; Okunade, A.L.; Klein, S. Adipose tissue monomethyl branched-chain fatty acids and insulin sensitivity: Effects of obesity and weight loss. *Obesity* 2015, 23, 329–334. [CrossRef]

12. Mika, A.; Stepnowski, P.; Kaska, L.; Proczyk, M.; Wisniewski, P.; Sledzinski, M.; Sledzinski, T. A comprehensive study of serum odd- and branched-chain fatty acids in patients with excess weight. *Obesity* 2016, 24, 1669–1676. [CrossRef]

13. Pakiet, A.; Wilczynski, M.; Rostkowska, O.; Korczynska, J.; Jablonska, P.; Kaska, L.; Proczyk-Stepaniak, M.; Sokoczek, E.; Stepnowski, P.; Magkos, F.; et al. The Effect of One Anastomosis Gastric Bypass on Branched-Chain Fatty Acid and Branched-Chain Amino Acid Metabolism in Subjects with Morbid Obesity. *Obes. Surg.* 2019, 30, 304–312. [CrossRef] [PubMed]

14. Yan, Y.; Wang, Z.; Wang, D.; Lawrence, P.; Wang, X.; Kothapalli, K.S.D.; Greenwald, J.; Liu, R.; Park, H.G.; Brenna, J.T. BCFA-enriched vernix-monoacylglycerol reduces LPS-induced inflammatory markers in human enterocytes in vitro. *Pediatr. Res.* 2018, 83, 874–879. [CrossRef] [PubMed]

15. Ran-Ressler, R.R.; Khalova, L.; Arganbright, K.M.; Adkins-Rieck, C.K.; Jouni, Z.E.; Koren, O.; Ley, R.; Brenna, J.T.; Dvorak, B. Branched chain fatty acids reduce the incidence of necrotizing enterocolitis and alter gastrointestinal microbial ecology in a neonatal rat model. *PLoS ONE* 2011, 6, e29032. [CrossRef] [PubMed]

16. Yan, Y.; Wang, Z.; Greenwald, J.; Kothapalli, K.; Park, H.; Liu, R.; Mendralla, E.; Lawrence, P.; Wang, X.; Brenna, J. BCFA suppresses LPS induced IL-8 mRNA expression in human intestinal epithelial cells. *Prostaglandins Leukot. Essent. Fat. Acids.* 2017, 116, 27–31. [CrossRef]
17. Kawai, T.; Autieri, M.V.; Scala, R. Adipose tissue inflammation and metabolic dysfunction in obesity. *Am. J. Physiol.-Cell Physiol.* 2021, 320, C375–C391. [CrossRef]

18. Zatterale, F.; Longo, M.; Naderi, J.; Raciti, G.A.; Desiderio, A.; Miele, C.; Beguinot, F. Chronic Adipose Tissue Inflammation Linking Obesity to Insulin Resistance and Type 2 Diabetes. *Front. Physiol.* 2020, 10, 1607. [CrossRef]

19. Wu, H.; Ballantyne, C.M. Metabolic Inflammation and Insulin Resistance in Obesity. *Circ. Res.* 2020, 126, 1549–1564. [CrossRef]

20. Harpsøe, M.C.; Basit, S.; Andersson, M.; Nielsen, N.M.; Frisch, M.; Wohlfahrt, J.; Nohr, E.A.; Linneberg, A.; Jess, T. Body mass index and risk of autoimmune diseases: A study within the Danish National Birth Cohort. *Int. J. Epidemiol.* 2014, 43, 843–855. [CrossRef]

21. El-Mikkawy, D.M.E.; El-Sadek, M.A.; El-Badawy, M.A.; Samaha, D. Circulating level of interleukin-6 in relation to body mass index and risk of autoimmune diseases: A study within the Danish National Birth Cohort. *Int. J. Epidemiol.* 2014, 43, 843–855. [CrossRef]

22. Matsuzaka, T.; Shimano, H. Elovl6: A new player in fatty acid metabolism and insulin sensitivity. *J. Mol. Med.* 2015, 93, 943–952. [CrossRef]

23. Matsuzaka, T.; Shimano, H. Elovl6: A new player in fatty acid metabolism and insulin sensitivity. *Adv. Nutr.* 2020, 11, 524–530. [CrossRef]

24. Vicennati, V.; Vottero, A.; Friedman, C.; Papanicolaou, D. Hormonal regulation of interleukin-6 production in human adipocytes. *Endocrinology* 2009, 150, 6015–6025. [CrossRef]

25. Eissing, L.; Scherer, T.; Tödter, K.; Knippschild, U.; Greve, J.W.; Buurman, W.A.; Pinnschmidt, H.O.; Rensen, S.S.; Wolf, A.M.; et al. Crucial role of a long-chain fatty acid elongase, Elovl6, in obesity-induced insulin resistance. *Acta Diabetol.* 2019, 56, 171–179. [CrossRef]

26. Wu, H.; Ballantyne, C.M. Metabolic Inflammation and Insulin Resistance in Obesity. *Circ. Res.* 2020, 126, 1549–1564. [CrossRef]

27. Zareie, R.; Yuzbashian, E.; Rahimi, H.; Asghari, G.; Zarkesh, M.; Hedayati, M.; Djazayery, A.; Movahedi, A.; Mirmiran, P.; Khalaj, A.; et al. Dietary fat content and adipose triglyceride lipase and hormone-sensitive lipase gene expressions in adults’ subcutaneous and visceral fat tissues. *Prostaglandins Leukot. Essent. Fat. Acids* 2021, 165, 102244. [CrossRef] [PubMed]

28. Le Lay, S.; Lefrere, I.; Trautwein, C.; Dugail, I.; Krief, S. Insulin and Sterol-regulatory Element-binding Protein-1c (SREBP-1C) Regulation of Gene Expression in 3T3-L1 Adipocytes: Identification of ccaat/enhancer-binding protein β as an SREBP-1C target. *J. Biol. Chem.* 2002, 277, 35625–35634. [CrossRef] [PubMed]

29. Bartelt, A.; et al. De novo lipogenesis in human fat and liver is linked to ChREBP-β and metabolic health. *Nat. Commun.* 2013, 4, 1528. [CrossRef] [PubMed]

30. Sekiya, M.; Yahagi, N.; Matsuzaka, T.; Takeuchi, Y.; Nakagawa, Y.; Takahashi, H.; Okazaki, H.; Iizuka, Y.; Ohashi, K.; Gotoda, T.; et al. SREBP-1-independent regulation of lipogenic gene expression in adipocytes. *J. Lipid Res.* 2007, 48, 1581–1591. [CrossRef]

31. Zetterale, F.; Longo, M.; Naderi, J.; Raciti, G.A.; Desiderio, A.; Miele, C.; Beguinot, F. Chronic Adipose Tissue Inflammation Linking Obesity to Insulin Resistance and Type 2 Diabetes. *Front. Physiol.* 2020, 10, 1607. [CrossRef]

32. Wohlfahrt, J.; Nohr, E.A.; Linneberg, A.; Jess, T. Body mass index and risk of autoimmune diseases: A study within the Danish National Birth Cohort. *Int. J. Epidemiol.* 2014, 43, 843–855. [CrossRef]

33. Matsuzaka, T.; Shimano, H. Elovl6: A new player in fatty acid metabolism and insulin sensitivity. *J. Mol. Med.* 2015, 93, 943–952. [CrossRef]

34. Matsuzaka, T.; Shimano, H. Elovl6: A new player in fatty acid metabolism and insulin sensitivity. *Nutrients* 2022, 14, 8, 7379–7396. [CrossRef]

35. Vicennati, V.; Vottero, A.; Friedman, C.; Papanicolaou, D. Hormonal regulation of interleukin-6 production in human adipocytes. *Endocrinology* 2009, 150, 6015–6025. [CrossRef]

36. Vicennati, V.; Vottero, A.; Friedman, C.; Papanicolaou, D. Hormonal regulation of interleukin-6 production in human adipocytes. *Endocrinology* 2009, 150, 6015–6025. [CrossRef]

37. Zetterale, F.; Longo, M.; Naderi, J.; Raciti, G.A.; Desiderio, A.; Miele, C.; Beguinot, F. Chronic Adipose Tissue Inflammation Linking Obesity to Insulin Resistance and Type 2 Diabetes. *Front. Physiol.* 2020, 10, 1607. [CrossRef]
42. Ganapathi, M.K.; May, L.T.; Schultz, D.; Brabeneck, A.; Weinstein, J.; Sehgal, P.B.; Kushner, I. Role of interleukin-6 in regulating synthesis of C-reactive protein and serum amyloid A in human hepatoma cell lines. Biochem. Biophys. Res. Commun. 1988, 157, 271–277. [CrossRef]
43. Lieb, D.C.; Brotman, J.J.; Hatcher, M.A.; Aye, M.S.; Cole, B.K.; Haynes, B.A.; Wohlgemuth, S.D.; Fontana, M.A.; Beydoun, H.; Nadler, J.L.; et al. Adipose tissue 12/15 lipoxygenase pathway in human obesity and diabetes. J. Clin. Endocrinol. Metab. 2014, 99, E1713–E1720. [CrossRef] [PubMed]
44. Cole, B.K.; Morris, M.A.; Grzesik, W.; Leone, K.A.; Nadler, J.L. Adipose tissue-specific deletion of 12/15-lipoxygenase protects mice from the consequences of a high-fat diet. Mediat. Inflamm. 2012, 2012, 851796. [CrossRef] [PubMed]
45. Ivanov, I.; Kuhn, H.; Heydeck, D. Structural and functional biology of arachidonic acid 15-lipoxygenase-1 (ALOX15). Biochem. Biophys. Res. Commun. 2010, 403, 485–490. [CrossRef]
46. Fain, J.N.; Leffler, C.W.; Cowan, G.S.; Buffington, C.; Pouncey, L.; Bahouth, S.W. Stimulation of leptin release by arachidonic acid 20–22:0 in human visceral adipose tissue in vitro. J. Nutr. Biochem. 2003, 14, 141–148. [CrossRef]
47. Higgs, G.; Salmon, J.; Spayne, J. The inflammatory effects of hydroperoxy and hydroxy acid products of arachidonate lipoxygenase 12HETE and 12HETE-lysine. Biochim. Biophys. Acta 2000, 1472–1480. [CrossRef] [PubMed]
48. Sears, D.D.; Miles, P.D.; Chapman, J.; Ofrecio, J.M.; Almazan, F.; Thapar, D.; Miller, Y.I. 12/15-lipoxygenase is required for the early onset of high fat diet-induced adipose tissue inflammation and insulin resistance in mice. PLoS ONE 2009, 4, e7250. [CrossRef]
49. Tian, R.; Zuo, X.; Jaoude, J.; Mao, F.; Colby, J.; Shureiqi, I. ALOX15 as a suppressor of inflammation and cancer: Lost in the link. [CrossRef] [PubMed]
50. Chakrabarti, S.; Cole, B.; Wen, Y.; Keller, S.R.; Carter, J.D.; Yang, Z.; Smith, K.M.; Wu, R.; Bevard, M.H.; et al. 12-Lipoxygenase-knockout mice are resistant to inflammatory effects of obesity induced by Western diet. Am. J. Physiol. Endocrinol. Metab. 2008, 295, E1065–E1075. [CrossRef] [PubMed]
51. Vellaisamy, R.; Sahay, D.; Yamada, K.; Liu, S.; Liao, Q.; Xiao, S.; Koc, E.; Ehrlich, S.A. et al. Peroxynitrite formation in adipose tissue and diet-induced obesity. PLoS ONE 2012, 7, e48171. [CrossRef] [PubMed]
52. Wajchenberg, B.L. Subcutaneous and Visceral Adipose Tissue: Their Relation to the Metabolic Syndrome. Am. J. Clin. Nutr. 2001, 74, 3115. [CrossRef] [PubMed]
53. Chan, P.-C.; Liao, M.-T.; Hsieh, P.-S. The Dualistic Effect of COX-2-Mediated Signaling in Obesity and Insulin Resistance. Prostaglandins Other Lipid Mediat. 2017, 132, 77–83. [CrossRef]
54. Chan, P.-C.; Hsiao, F.C.; Chang, H.M.; Wabitsch, M.; Hsieh, P.-S. Importance of adipocyte cyclooxygenase-2 and prostaglandin E2-prostaglandin E receptor 3 signaling in 3T3-L1 adipocytes. Obesity 2009, 17, 1657–1663. Available online: https://pubmed.ncbi.nlm.nih.gov/19521344/ (accessed on 2 November 2021).
55. Fain, J.N.; Leffler, C.W.; Cowan, G.S.; Buffington, C.; Pouncey, L.; Bahouth, S.W. Stimulation of leptin release by arachidonic acid 20–22:0 in human visceral adipose tissue in vitro. J. Nutr. Biochem. 2003, 14, 141–148. [CrossRef] [PubMed]
56. Nunemaker, C.S.; Chen, M.; Pei, H.; Kimble, S.D.; Keller, S.R.; Carter, J.D.; Yang, Z.; Smith, K.M.; Wu, R.; Bevard, M.H.; et al. 12-Lipoxygenase-knockout mice are resistant to inflammatory effects of obesity induced by Western diet. Am. J. Physiol. Endocrinol. Metab. 2008, 295, E1065–E1075. [CrossRef] [PubMed]
57. Nunemaker, C.S.; Chen, M.; Pei, H.; Kimble, S.D.; Keller, S.R.; Carter, J.D.; Yang, Z.; Smith, K.M.; Wu, R.; Bevard, M.H.; et al. 12-Lipoxygenase-knockout mice are resistant to inflammatory effects of obesity induced by Western diet. Am. J. Physiol. Endocrinol. Metab. 2008, 295, E1065–E1075. [CrossRef] [PubMed]
58. Ran-Ressler, R.R.; Bae, S.; Lawrenc, P.; Wang, D.; Brenna, J.T. Branched-chain fatty acid content of foods and estimated intake in the USA. Br. J. Nutr. 2014, 112, 565–572. [CrossRef] [PubMed]
59. Berndt, J.; Kovacs, P.; Ruschke, K.; Klötting, N.; Fasshauer, M.; Schön, M.R.; Körner, A.; Stumvoll, M.; Blüher, M. Fatty acid synthase gene expression in human adipose tissue: Association with obesity and type 2 diabetes. Diabetologia 2007, 50, 1472–1480. [CrossRef] [PubMed]
60. Ashaolu, T.J.; Saibandith, B.; Yupanqui, C.T.; Wichienchot, S. Human colonic microbiota modulation and branched chain fatty acids production affected by soy protein hydrolysate. Int. J. Food Sci. Technol. 2019, 54, 141–148. [CrossRef]
61. Caraballo, S.C.G.; Combair, T.M.; Houten, S.M.; Dejong, C.H.; Lamers, W.H.; Koehler, S.E. High-protein diets prevent steatosis and induce hepatic accumulation of monomethyl branched-chain fatty acids. J. Nutr. Biochem. 2014, 25, 1263–1274. [CrossRef]
62. Wajchenberg, B.L. Subcutaneous and Visceral Adipose Tissue: Their Relation to the Metabolic Syndrome. Endocr. Rev. 2000, 21, 672–738. [CrossRef]
63. Berndt, J.; Kovacs, P.; Ruschke, K.; Klötting, N.; Fasshauer, M.; Schön, M.R.; Körner, A.; Stumvoll, M.; Blüher, M. Fatty acid synthase gene expression in human adipose tissue: Association with obesity and type 2 diabetes. Diabetes 2007, 50, 1472–1480. [CrossRef] [PubMed]
64. Ashaolu, T.J.; Saibandith, B.; Yupanqui, C.T.; Wichienchot, S. Human colonic microbiota modulation and branched chain fatty acids production affected by soy protein hydrolysate. Int. J. Food Sci. Technol. 2019, 54, 141–148. [CrossRef] [PubMed]
65. Caraballo, S.C.G.; Combair, T.M.; Houten, S.M.; Dejong, C.H.; Lamers, W.H.; Koehler, S.E. High-protein diets prevent steatosis and induce hepatic accumulation of monomethyl branched-chain fatty acids. J. Nutr. Biochem. 2014, 25, 1263–1274. [CrossRef] [PubMed]
66. Wajchenberg, B.L. Subcutaneous and Visceral Adipose Tissue: Their Relation to the Metabolic Syndrome. Endocr. Rev. 2000, 21, 672–738. [CrossRef] [PubMed]