DOI: 10.1002/vms3.383

ORIGINAL ARTICLE

WILEY

Nutmeg extract potentially alters characteristics of white adipose tissue in rats

Ronny Lesmana[1,2,3] | Melisa Siannoto[4] | Gaga I. Nugraha[5] | Hanna Goenawan[1,2] | Astrid K. Feinisa[4,6] | Yuni S. Pratiwi[1,2] | Fifi Veronica[7] | Vita M. Tarawan[1] | Susianti Susianti[3,8] | Unang Supratman[3,8]

[1]Physiology Division, Department of Biomedical Sciences, Faculty of Medicine, Universitas Padjadjaran, Bandung, West Java, Indonesia

[2]Biological Activity Division, Central Laboratory, Universitas Padjadjaran, Bandung, West Java, Indonesia

[3]Center of Excellence in Higher Education for Pharmaceutical Care Innovation, Universitas Padjadjaran, Bandung, West Java, Indonesia

[4]Graduate Program of Anti Aging and Aesthetics Medicine, Faculty of Medicine, Universitas Padjadjaran, Bandung, West Java, Indonesia

[5]Division of Biochemistry and Biomolecular, Department of Biomedical Sciences, Faculty of Medicine, Universitas Padjadjaran, Bandung, West Java, Indonesia

[6]Division of Cell Biology, Department of Biomedical Sciences, Faculty of Medicine, Universitas Padjajaran, Bandung, West Java, Indonesia

[7]Anatomy Division, Department of Biomedical Sciences, Faculty of Medicine, University of Padjadjaran, Bandung, West Java, Indonesia

[8]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Bandung, West Java, Indonesia

Correspondence
Ronny Lesmana, Physiology Division, Department of Biomedical Sciences, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia.
Email: ronny@unpad.ac.id

Funding information
Direktorat Jenderal Pendidikan Tinggi

Abstract

Background: Browning of white adipose tissue (WAT) is a promising approach to obesity treatment. During browning, WAT transforms into beige adipose tissue through stimulation of the peroxisome proliferator activated receptor γ (PPARγ). Nutmeg, one of the Indonesian herbs, reportedly has dual roles as a PPARα/γ partial agonist. Even though nutmeg has been traditionally used in body weight reduction, there is limited information regarding the potential role of nutmeg in browning of WAT.

Objectives: In this study, we explored the effect of nutmeg seed extract (NuSE) as a potential inductor of WAT browning.

Methods: Twelve male Wistar rats, 5–6 weeks old, were divided into control and nutmeg groups. The rats in nutmeg group were given NuSE for 12 weeks by oral gavage. After 12 weeks, the rat's inguinal WAT and brown adipose tissue (BAT) were collected, weighed and stored at − 80°C until use.

Results: We observed that even though NuSE did not reduce the final body weight, it significantly reduced body weight gain. NuSE also increased protein levels of peroxisome proliferator activated receptor γ coactivator 1α (PGC-1α) and uncoupling protein 3 (UCP3) significantly and tended to increase UCP2 and UCP1 levels. Furthermore, NuSE induced macroscopic and microscopic morphological changes of inguinal WAT, marked by significantly increased adipocyte numbers and decreased adipocyte size.

Ronny Lesmana and Melisa Siannoto are co-first author.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
© 2020 The Authors. *Veterinary Medicine and Science* Published by John Wiley & Sons Ltd

Conclusions: Even though NuSE did not increase UCP1 significantly, it potentially alters inguinal WAT characteristics and leads to browning through PGC-1α and UCP3 induction. However, UCP3's specific mechanism in WAT browning remains unclear. Our findings could contribute to obesity treatment in the future.

KEYWORDS
browning, nutmeg, obesity, PGC-1α, UCP1, UCP3

1 | INTRODUCTION

The worldwide prevalence of obesity has almost tripled since 1975 (World Health Organization, 2018). In 2016, the World Health Organization (WHO) estimated that 1.9 billion adults were overweight, and 650 million were obese (World Health Organization, 2018). Obesity occurs when consumed energy chronically exceeds expended energy, so the excess energy is stored in adipose tissue, especially in white adipose tissue (WAT) (Rui, 2017). WAT accumulation increases adiposity, causes local inflammation and leads to several comorbidities (Steinbeck et al., 2018). Thus, the rapid growth in obesity prevalence, along with its many comorbidities and high mortality rate, highlights the importance of early detection and management of obesity.

One of the promising approaches in obesity management is by increasing thermogenesis. Thermogenesis contributes around 10% of total energy expenditure (Abdelaal et al., 2017; Solas et al., 2016) and mainly occurs in brown adipose tissue (BAT) and skeletal muscle (Periasamy et al., 2017). Several studies reported that another type of adipose tissue, known as beige adipose tissue, also contributes to thermogenesis (Fuller-Jackson & Henry, 2018; Ohno et al., 2012; Petrovic et al., 2010; Ravussin et al., 2014; Richard et al., 2017). Under certain stimulations, beige adipose tissue can have similar characteristics and functions with BAT through a process known as browning (Okla et al., 2017). Despite recent discoveries that BAT can be found in limited amounts in adults, its quantity and thermogenic effect decrease significantly with age (Wu et al., 2013). Thus, the thermogenesis that occurs in beige adipose tissue has a beneficial effect on obesity management, especially in adults. A deeper understanding of WAT browning, along with its inductors, may lead to new therapeutics for obesity. Several well-studied inductors of WAT browning are cold (Ravussin et al., 2014), β3-adrenergic agonist (β3AR) (Richard et al., 2017), peroxisome proliferator activated receptor γ (PPARγ) agonist (Ohno et al., 2012; Petrovic et al., 2010), irisin (Boström et al., 2012) and fibroblast growth factor 21 (FGF21) (Fisher et al., 2012).

There are several WAT browning markers, such as uncoupling protein 1 (UCP1), peroxisome proliferator activated receptor γ coactivator 1α (PGC-1α), CD137, transmembrane protein 26 (Tmeme26) and many more (Bargut et al., 2017). At first, UCP1 was considered the only uncoupling protein to be involved in the thermogenesis mechanism. After the discovery of other UCPs that have high homology with UCP1 (namely UCP2 and UCP3), several studies have indicated that these UCPs might be involved in thermogenesis (Cannon & Nedergaard, 2010). Previous studies reported that UCP2 might be involved in thermogenesis in BAT (Boss et al., 1997; Caron et al., 2017), even though it is still unclear whether this UCP is also involved in browning. The expression of *Ucp2*, along with *Ucp1*, reportedly increased after cold stimulation, indicating that this protein might be involved in thermogenesis (Boss et al., 1997). Similar to UCP2, UCP3's role in thermogenesis and browning of WAT is also undetermined. In the beginning, UCP3 was not considered significant in thermogenesis and browning due to its limited expression in adipose tissues (Sluse & Scatena, 2012). However, several studies reported that *Ucp3* expression in adipose tissue increased after certain stimulations, indicating that *Ucp3* might be involved in browning of WAT and thermogenesis (Gong et al., 1997; Lin et al., 2017).

The pharmacological agonists of PPARγ and β3AR induce significant browning of WAT, but due to their side effects, their long-term use as obesity treatment is not recommended (Ohno et al., 2012; Petrovic et al., 2010; Richard et al., 2017). One of the potential PPARγ agonists, Rosiglitazone, reportedly induces browning of WAT and increases mitochondrial biogenesis. However, it can cause serious side effects, such as myocardium infarction and heart failure (Nissen & Wolski, 2010; Ohno et al., 2012), so it is not recommended for long-term obesity treatment. Therefore, the inventions of natural nutraceuticals that can induce the browning process but with fewer side effects, become more important. Several nutraceuticals have been proven to induce browning of WAT through several mechanisms (Siannoto et al., 2020). Nutmeg (*Myristica fragrans* Houtt.), one of Indonesia's herbal medicines that acts as PPARα/γ partial agonist, is consider to have ability to induce browning of WAT (Lestari et al., 2012).

Nutmeg is one of the nutraceuticals that have health benefits. Several studies reported that nutmeg extract has potential effects against diabetes and dyslipidemia due to its role as a dual agonist of PPARα/γ, even though it has less effect compared with full agonists (Lestari et al., 2012, 2019). Another study also reported that administering nutmeg extract for 12 weeks upregulated *Pgc-1α* expression in the rat's brain, indicating mitochondrial biogenesis stimulation (Veronica et al., 2018). A 2018 study also reported that nutmeg extract could decrease the body weight of old rats after 10 weeks of administration (Pratiwi et al., 2018). Here, we study the effects of nutmeg seed extract (NuSE) in browning of WAT. We also analyse the role of UCP1 and its homologs (UCP2 and UCP3), along with

PGC-1α, which may be correlated with the changes to WAT's macroscopic and microscopic characteristics.

2 | MATERIALS AND METHODS

2.1 | Nutmeg extract (NuSE)

To minimize hallucinogenic and hepatotoxicity effects, safrole and myristicin-free NuSE were used. The chemical analysis and phytochemicals screening of NuSE can be seen in Tables S1 and S2. Nutmeg seeds were taken from Maluku and West Java Island. The extraction and purification of nutmeg in this study were adapted from our previous study (Pratiwi et al., 2018).

2.2 | Ethical statement

Animal procedures were approved and carried out according to the guidelines of the Animal Ethics Committee of Universitas Padjadjaran Bandung, Indonesia (Ethical Approval No. 1080/UN6. KEP/EC/2019).

2.3 | Animal model

Male Wistar rats at 5–6 weeks-old (body weight around 200 g; $n = 12$) were brought from PT Bio Farma, Bandung, Indonesia. Rats were kept at 24°C, 55% relative humidity and a 12-hr light-dark cycle, with a standard diet (the diet composition can be seen in Table S3) and water ad libitum. After acclimatization for a week, rats were divided randomly into two groups: control and treatment (NuSE). The control group was given pulvis gummi arabicum (PGA) 2%, and the treatment group was given 8.1 mg/kg body weight/day NuSE (the specific dose was counted for each rat) (Pratiwi et al., 2018). All the drugs were given to the animals by oral gavage for 12 weeks. Body weight and food intake were measured weekly. After 12 weeks of protocol, animals were sacrificed using the CO_2 chamber. The inguinal and interscapular fat was carefully dissected, weighed and stored at −80°C until use. The inguinal WAT was used instead of epididymal WAT because a previous study reported that WAT was more challenging to transform into beige adipose tissue due to its lower *Prdm16* expression (Seale et al., 2011).

2.4 | Histological analysis

The dissected adipose tissues were fixed in 4% paraformaldehyde and embedded in paraffin. The tissues were cut at 6–8 μm thickness and stained with Hematoxylin-Eosin (HE). Microscope figures were taken using Zeiss Imager.Z2 microscope. The adipose tissue size and the total number per microscopic field were counted and analysed using Image J software.

2.5 | Western blot analysis

The dissected adipose tissues were weighed (25 mg ± 1), homogenized, lysed in 100 μl RIPA buffer, and combined with a sample buffer (with 1:1 ratio). Then, the lysate protein sample was denatured in the heating block at 95°C for 5 min. Equal amounts of samples (10 μg/lane) were separated using SDS-PAGE, transferred to a nitrocellulose membrane for 1 hr at room temperature, and then blocked overnight at 4°C in 1% blocking skim milk in phosphate-buffered saline buffer with 0.1% Tween 20 (PBST). Immunoblotting was performed using a rabbit monoclonal UCP1 (#U6382) and UCP3 (#U7757) from Sigma Aldrich; UCP2 (#89326) from Cell Signaling, and mouse monoclonal PGC-1α (PA5-38022) and β-actin (MA5-15739) from Thermo Scientific with dilution 1:1,000 in PBST buffer. Secondary antibody anti-mouse (C90130-03) and anti-rabbit (C81106-06) were purchased from RND with dilution 1:15,000 in PBST buffer. Beta actin was used as an internal control. The membranes were developed using infrared enriched secondary antibody reagent (LI-COR), imaged using LI-COR Odyssey CLX, and the intensities of the band were determined using LI-COR software.

2.6 | Statistical analysis

All data were expressed as mean ± standard error of the mean. Statistical analysis was conducted using SPSS software version 25. Data were analyzed with One-Way Analysis of Variance and continued with post hoc test (least significant difference test if the data distribution was normal and homogenous and Mann–Whitney test if data distribution was not normal and/or homogenous). Statistical significance was designated at $p < 0.05$.

3 | RESULTS

3.1 | NuSE did not significantly reduce final body weight, but did reduce body weight gain

After 12-week-administration of NuSE, we observed different body weight changes between the control and NuSE group (Figure 1a). At the end of our study, there were no significant differences in body weight between the control and NuSE groups (Figure 1b). However, the Δ body weight of the control and NuSE groups showed significant differences in weeks 3, 6, 9 and 12 (Figure 1c). Overall, rats in the control group gained more weight than the NuSE group (Figure 1d).

3.2 | NuSE increased number of adipocytes and decreased adipocytes size in inguinal WAT

NuSE stimulated morphological changes of inguinal WAT, similar to beige adipose tissue appearance, both from macroscopic (Figure 2a) and microscopic views (Figure 2b). NuSE significantly increased

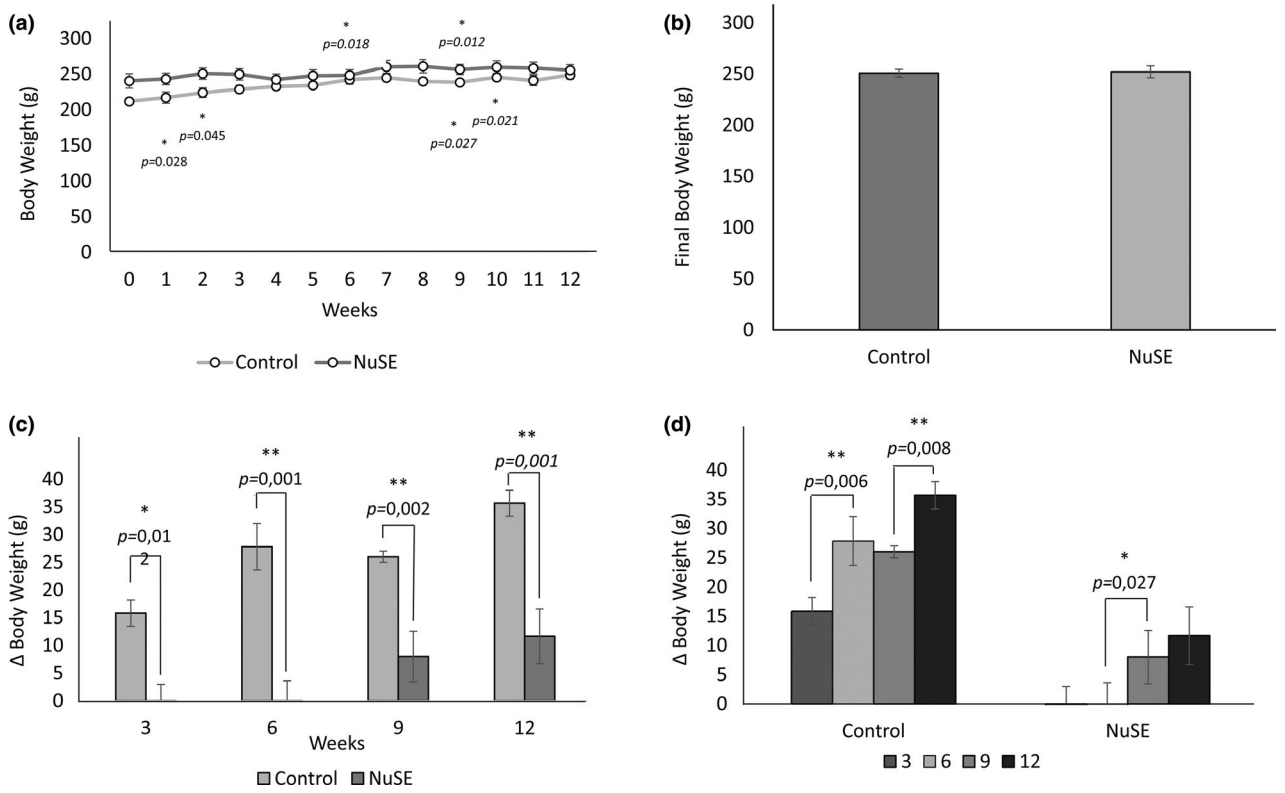

FIGURE 1 NuSE (a) caused several changes in body weight both in control and NuSE groups ($n = 6$); (b) at the end of the treatment, rats in the control group showed a significant increase in body weight; (c) the changes of body weight between control and nutmeg groups showed significant differences in weeks 3, 6, 9 and 12; (d) rats in the control group showed more significant increases in body weight compared to NuSE group; $^{*}p < .05$; $^{**}p < .01$

the number of adipocytes (Figure 2c) and reduced adipocyte size (Figure 2d). Meanwhile, we found no significant differences between the weight of inguinal WAT and BAT in the control and nutmeg groups (Figure 2e).

3.3 | NuSE significantly increased protein levels of UCP3 and PGC-1α and had tendencies to increase UCP1 and UCP2 levels

NuSE significantly increased the protein levels of UCP3 and PGC-1α in the NuSE group (Figure 3a and b) by 1.5-folds compared to the control group and had tendencies to increase protein levels of UCP1 ($p = 0.062$) and UCP2 ($p = 0.058$).

4 | DISCUSSION

PPARγ agonists have been reported to activate browning of WAT (Ohno et al., 2012; Petrovic et al., 2010). Among many known natural PPARγ agonists, nutmeg has been reported to have dual roles as PPARα/γ agonists (Lestari et al., 2012).

In this study, we found that both rats in control and NuSE groups showed increased body weight, even though the body weight gain in the NuSE group was less than the control group (Figure 1c and d). These results were different compared to our previous study, in which we found that the 12-week-administration of NuSE can significantly decrease body weight in old rats (Pratiwi et al., 2018). The difference in rat's age in our present and previous study may determine the baseline level of metabolism and alter several physiological factors, such as hormonal level, inflammatory responses and level of oxidative stress (Bratic & Larsson, 2013). Thus, we suspect that nutmeg can have a different effect on the body weight of young and old rats.

NuSE also stimulated some microscopic morphological changes of inguinal WAT, marked by the smaller size of adipocytes and larger number of adipocytes (Figure 2b). The average number of adipocytes in one microscopic field in NuSE group was significantly increased by 120.96%, and the adipocyte size was significantly decreased by 69.23% compared to inguinal WAT of the control group (Figure 2c and d). These changes indicated that NuSE might stimulate the characteristic changes of inguinal WAT to beige adipose tissue. These changes can also indicate lipolysis activation, in which there were small amount of lipid droplets histologically due to triglycerides breakdown (Schweiger et al., 2014). Therefore, we cannot exclude the possibility that histological changes in this study was a consequence of lipolysis activation.

In our study, we found that NuSE increased both PGC-1α and UCP1 protein levels (Figure 3a), even though the increase of UCP1 was less significant. UCP1 and PGC-1α are important browning

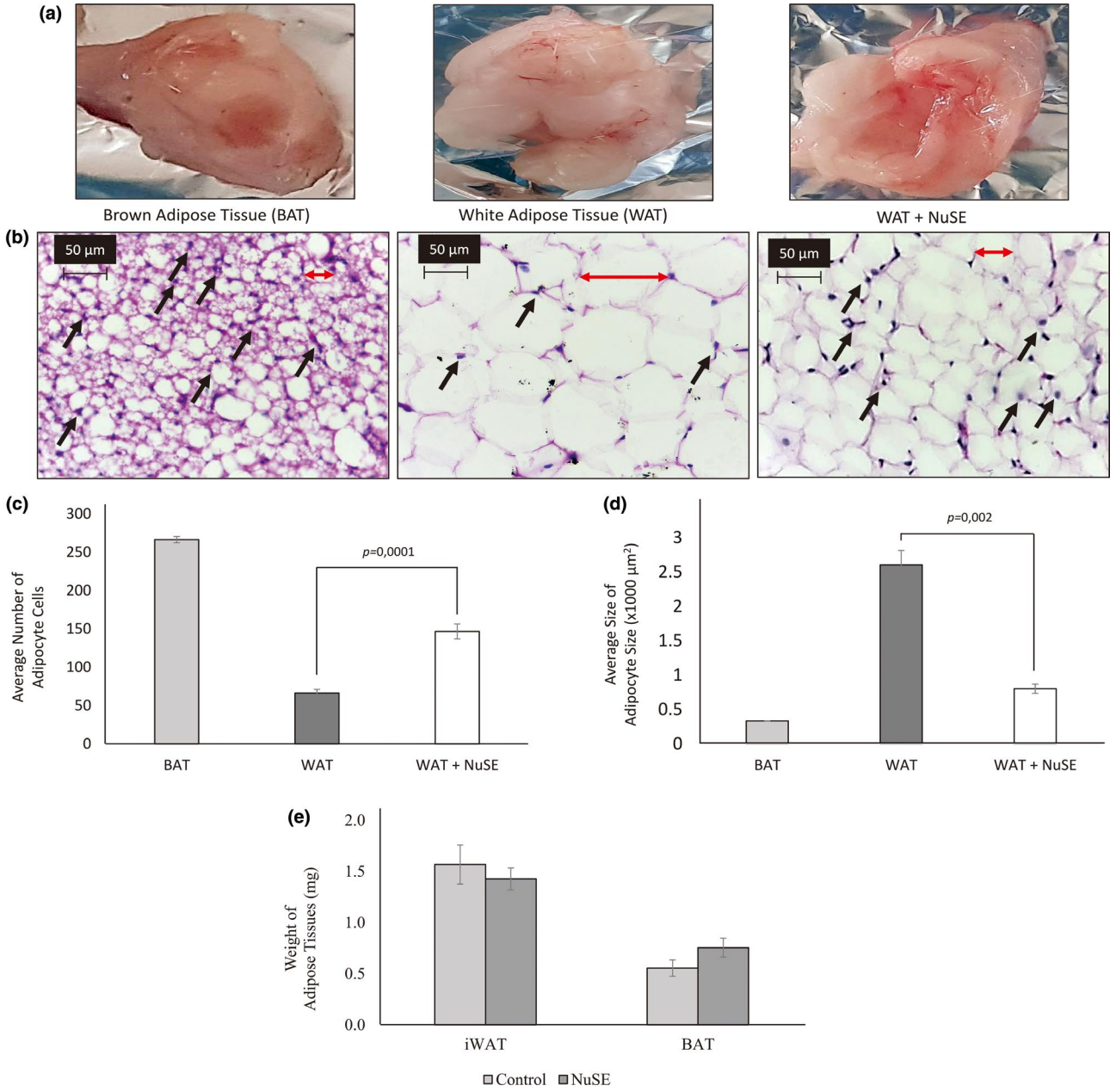

FIGURE 2 NuSE induced brown fat-like changes in iWAT ($n = 6$) both in (a) macroscopic view and (b) microscopic view of interscapular BAT, iWAT from the control group, iWAT from NuSE group with HE staining; histological analysis with 400 × magnification (scale bar = 50 µm) using Image J software (3 different sections were used for each sample) showed: (c) increase of adipocytes number in iWAT of NuSE groups and (d) decrease of adipocyte size in inguinal WAT of NuSE groups; (e) NuSE did not change the weight of iWAT and interscapular BAT from control and NuSE groups. Red arrows indicated the size of adipocyte; the black arrows indicated the nucleus

markers because UCP1 is the main protein that is involved in thermogenesis and PGC-1α is the master regulator of mitochondrial biogenesis. Previous studies have reported that PPARγ agonists can activate browning of WAT, marked by the increase of UCP1, PGC-1α and other BAT/beige adipocyte protein markers (Coelho et al., 2016; Ohno et al., 2012; Petrovic et al., 2010). These studies reported that, to initiate browning of WAT, the induction must be chronic (Petrovic et al., 2010) and use full PPARγ agonists (Ohno et al., 2012). A partial PPARγ agonist had minimal to no effect on browning of WAT (Ohno et al., 2012). A study by Fukui et al. have shown that even though the administration of weak PPARγ can increase *Ucp1* expression, but whether it also induced browning of WAT was still unclear (Fukui et al., 2000). Another study by Coelho et al. reported different results, in which a new partial PPARγ agonist can induce thermogenesis-related genes in visceral WAT of mice (Coelho et al., 2016). As nutmeg only had a partial effect as PPARα/γ agonist (Lestari et al., 2012), there is a possibility that its agonist effect or its binding to the receptor was not strong enough to increase UCP1 level significantly, but was enough to induce mitochondrial biogenesis (marked by the significant increase

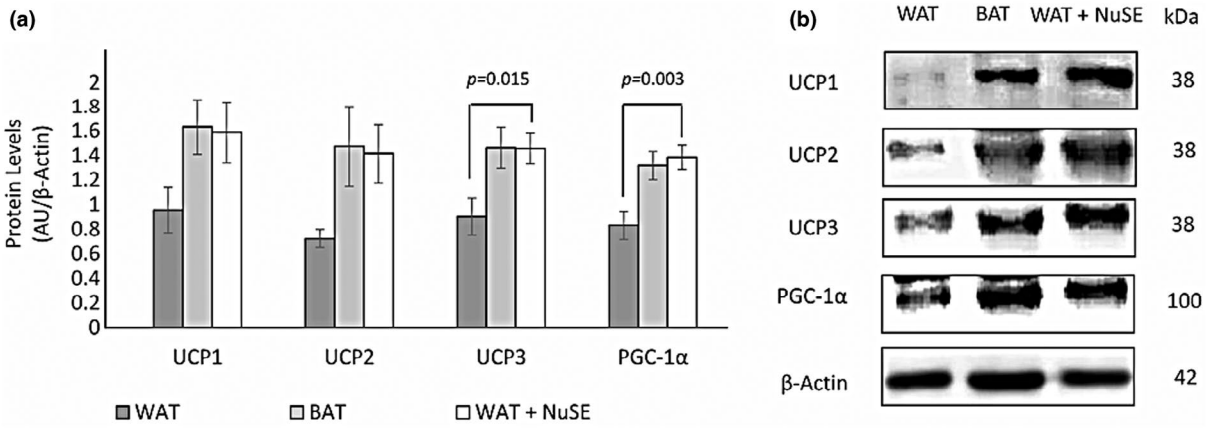

FIGURE 3 (a) NuSE significantly increased protein levels of UCP3 and PGC-1α and tended to increase UCP1 and UCP2 levels in young rats ($n = 6$); (b) representative western blots showed the increase of UCP1, UCP2, UCP3 and PGC-1α levels in NuSE group

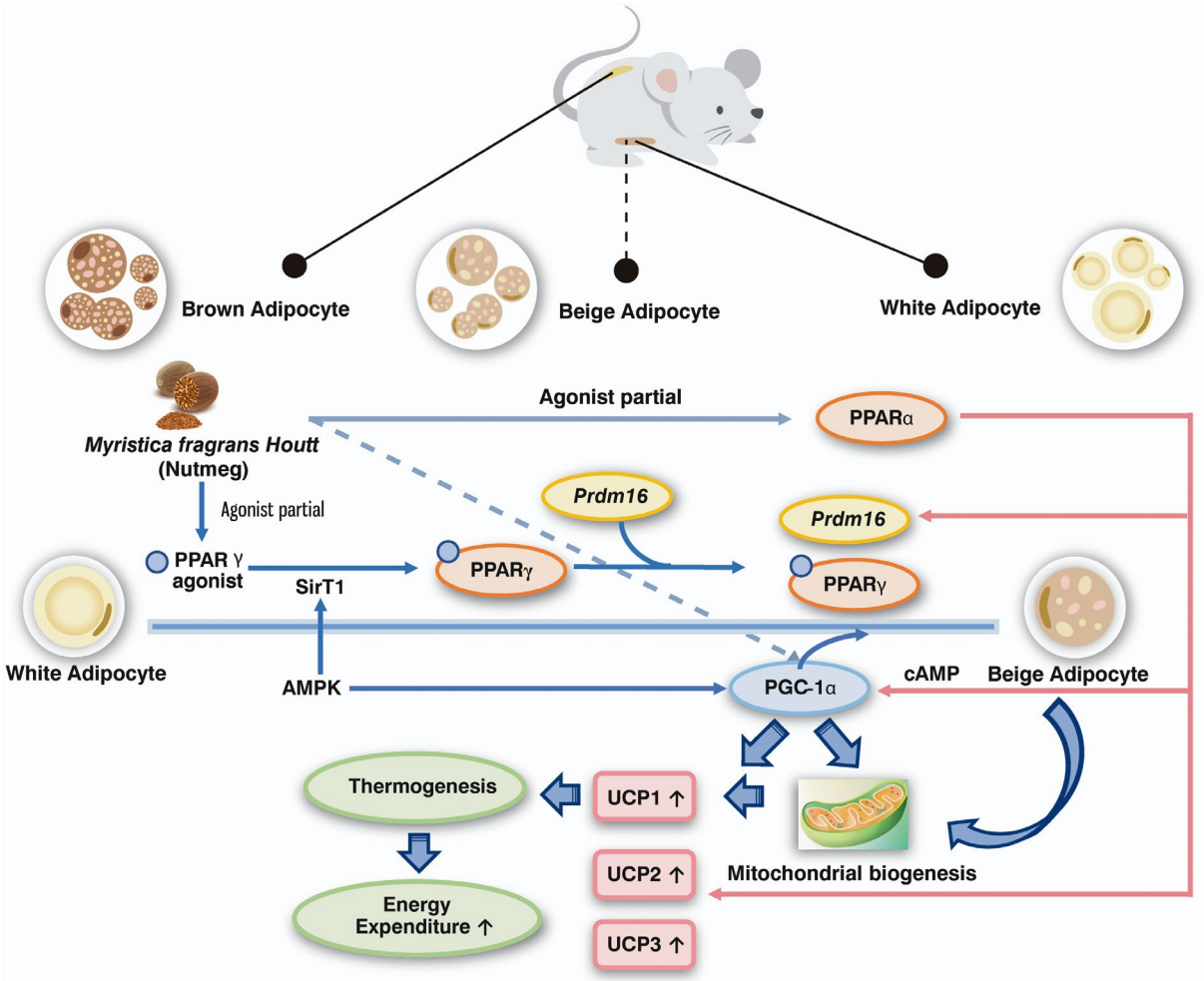

FIGURE 4 The proposed mechanisms of nutmeg in browning of WAT

of PGC-1α). Besides, in this study, we only used a single dose of nutmeg extract (the dose used in this study was based on previous studies (Pratiwi et al., 2018; Veronica et al., 2018)); thus, readjusting the dose could address more optimal browning processes. The role of nutmeg extract in inducing mitochondrial biogenesis in the rat's brain also has been reported (Veronica et al., 2018). The significant increase of PGC-1α protein level and histological changes showed that NuSE alters WAT characteristics. However, the non-significant increase of UCP1 protein level might indicate that other proteins are involved.

Interestingly, 12 weeks of NuSE administration resulted in significant increase of UCP3 protein level by 66.27% compared to the control groups (Figure 3a). UCP2 protein level was also observed to be elevated, the increase though was not statistically significant (Figure 3a). Even though several studies reported that UCP2 (Boss et al., 1997; Caron et al., 2017) and UCP3 (Gong et al., 1997; Lin et al., 2017) might have roles in thermogenesis, their roles in WAT browning is not well-established. Previous studies reported increases in UCP2 and UCP3 expressions after induction with weak PPARγ (Fukui et al., 2000) and PPARα (Nakatani et al., 2002) agonists. PPARγ and PGC-1α induce *Ucp3* expression in skeletal muscle (Marroquin, 2015). Drugs or substances can induce WAT browning or BAT differentiation, and will also induce gene expression of UCP3 in WAT (Villarroya et al., 2007). Thus, we suspect that the increased UCP2 and UCP3 levels may correlate with browning of WAT, even though the exact mechanism is still unclear. However, we cannot rule out the possibility that the increases were caused directly through the agonist effects of both PPARα/γ. Thus, further study is needed to confirm the role of UCP2 and UCP3 in the browning process and thermogenesis. We propose a potential mechanism of NuSE in inducing characteristic changes of inguinal WAT (Figure 4).

5 | CONCLUSION

NuSE alters both macroscopic and microscopic characteristics of inguinal WAT, marked by a significant increase of adipocyte number and decrease of adipocyte size. NuSE also stimulates the increases of UCP3 and PGC-1α protein levels and had tendencies to increase UCP1 and CP2 levels. These changes indicate that NuSE may potentially activate browning of WAT and in the future NuSE could contribute to bodyweight reduction and obesity management, especially by increasing the thermogenesis. However, further study is still needed.

ACKNOWLEDGEMENTS

The authors thank Keri Lestari, Rizky Abdulah and Titing Nurhayati for critically reviewed; Susianti, Nurul Assidiqiah and Canadia Ravelita for technical assistance during experiments. This research was supported by World Class Research -Indonesian Ministry of Research and Technology to Ronny Lesmana (2020-2023).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

AUTHOR CONTRIBUTION

ronny lesmana: Conceptualization; Data curation; Funding acquisition; Investigation; Methodology; Supervision; Validation; Writing-original draft; Writing-review & editing. **Melisa Siannoto:** Data curation; Formal analysis; Investigation; Project administration; Writing-original draft. **Gaga Irawan Nugraha:** Formal analysis; Methodology; Software; Supervision; Visualization; Writing-review & editing. **Hanna Goenawan:** Data curation; Formal analysis; Methodology; Supervision; Visualization; Writing-original draft; Writing-review & editing. **Astrid Khairani Feinisa:** Formal analysis; Investigation; Supervision; Validation; Visualization; Writing-review & editing. **Vita Muniarti Tarawan:** Conceptualization; Data curation; Investigation; Visualization. **Yuni Susanti Pratiwi:** Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Supervision; Visualization; Writing-review & editing. **Susianti Susianti:** Data curation; Investigation; Methodology; Project administration; Validation; Visualization. **Fifi Veronica:** Data curation; Methodology; Resources; Visualization. **Unang Supratman:** Formal analysis; Investigation; Methodology; Resources; Supervision; Visualization; Writing-review & editing.

AUTHOR'S CONTRIBUTIONS

Ronny Lesmana, Melisa Siannoto, Gaga Irawan Nugraha, Fifi Veronica participated in the design of the experiment, supervised the experiment and drafted the manuscript. Ronny Lesmana, Melisa Siannoto and Susianti are participated in conducting the experiment. Hanna Goenawan, Vita Muniarti Tarawan, Astrid Feinisa Khairani, Yuni Susanti Pratiwi, and Unang Supratman helped draft the manuscript and provided mentorship support.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/vms3.383.

DATA AVAILABILITY STATEMENT

The data of this study are available upon request.

ORCID

Ronny Lesmana https://orcid.org/0000-0002-7425-915X

REFERENCES

Abdelaal, M., le Roux, C. W., & Docherty, N. G. (2017). Morbidity and mortality associated with obesity. *Annals of Translational Medicine*, *5*(7), 161–174. https://doi.org/10.21037/atm.2017.03.107 https://doi.org/10.21037/atm.2017.03.107

Bargut, T. C. L., Souza-Mello, V., Aguila, M. B., & Mandarim-de-Lacerda, C. A. (2017). Browning of white adipose tissue: Lessons from experimental models. *Hormone Molecular Biology and Clinical Investigation*, *31*(1), 1–13. https://doi.org/10.1515/hmbci-2016-0051 https://doi.org/10.1515/hmbci-2016-0051

Boss, O., Samec, S., Dulloo, A., Seydoux, J., Muzzin, P., & Giacobino, J.-P. (1997). Tissue-dependent upregulation of rat uncoupling protein-2 expression in response to fasting or cold exposure. *FEBS Letters*, *412*(1), 111–114. https://doi.org/10.1016/S0014-5793(97)00755-2

Boström, P., Wu, J., Jedrychowski, M. P., Korde, A., Ye, L., Lo, J. C., Rasbach, K. A., Boström, E. A., Choi, J. H., Long, J. Z., Kajimura, S., Zingaretti, M. C, Vind, B. F, Tu, H, Cinti, S, Holjund, K, Gygi, S. P, & Spiegelman, B. M (2012). A PGC1-a-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature*, *481*(7382), 463–468.

Bratic, A., & Larsson, N. (2013). The role of mitochondria in aging. *Journal of Clinical Investigation*, *123*(3), 951–957. https://doi.org/10.1172/JCI64125 https://doi.org/10.1172/JCI64125

Cannon, B., & Nedergaard, J. (2010). Metabolic consequences of the presence or absence of the thermogenic capacity of brown adipose

tissue in mice (and probably in humans). *International Journal of Obesity*, *34*(S1), S7–S16. https://doi.org/10.1038/ijo.2010.177 https://doi.org/10.1038/ijo.2010.177

Caron, A., Labbé, S. M., Carter, S., Roy, M.-C., Lecomte, R., Ricquier, D., Picard, F., & Richard, D. (2017). Loss of UCP2 impairs cold-induced non-shivering thermogenesis by promoting a shift toward glucose utilization in brown adipose tissue. *Biochimie*, *134*, 118–126. https://doi.org/10.1016/j.biochi.2017.01.006 https://doi.org/10.1016/j.biochi.2017.01.006

Coelho, M. S., de Lima, C. L., Royer, C., Silva, J. B., Oliveira, F. C. B., Christ, C. G., Pereira, S. A., Bao, S. N., Lima, M. C. A., Pitta, M. G. R., Pitta, I. R., Neves, F. A. R., & Amato, A. A. (2016). GQ-16, a TZD-derived partial PPARγ agonist, induces the expression of thermogenesis-related genes in brown fat and visceral white fat and decreases visceral adiposity in obese and hyperglycemic mice. *PLoS One*, *11*(5), 1–20. https://doi.org/10.1371/journal.pone.0154310 https://doi.org/10.1371/journal.pone.0154310

Fisher, M., Kleiner, S., Douris, N., Fox, E. C., Mepani, R. J., Verdeguer, F., Wu, J., Kharitonenkov, A., Flier, J. S., Maratos-Flier, E., & Spiegelman, B. M. (2012). FGF21 regulates PGC-1 alpha and browning of white adipose tissues in adaptive thermogenesis. *Genes & Development*, *26*(3), 271–281.

Fukui, Y., Masui, S., Osada, S., Umesono, K., & Motojima, K. (2000). A new thiazolidinedione, NC-2100, which is a weak PPAR-gamma activator, exhibits potent antidiabetic effects and induces uncoupling protein 1 in white adipose tissue of KKAy obese mice. *Diabetes*, *49*(5), 759–767. https://doi.org/10.2337/diabetes.49.5.759 https://doi.org/10.2337/diabetes.49.5.759

Fuller-Jackson, J.-P., & Henry, B. A. (2018). Adipose and skeletal muscle thermogenesis: studies from large animals. *Journal of Endocrinology*, *273*(3), R99–R115. https://doi.org/10.1530/JOE-18-0090

Gong, D., He, Y., Karas, M., & Reitman, M. (1997). Uncoupling protein-3 is a mediator of thermogenesis regulated by thyroid hormone, beta 3 adrenergic agonist, and leptin. *Journal of Biological Chemistry*, *272*(39), 24129–24132..

Lestari, K., Diantini, A., Barliana, M. I., Achmad, T. H., Subarnas, A., Mutakin, Abdulah, R., Lesmana, R., & Hwang, J. K. (2019). Potential natural dual agonist PPAR α/γ -induced antidiabetic and anti-dyslipidemic properties of safrole-free nutmeg seed (Myristica fragrans Houtt) extract. *Journal of Natural Products*, *9*, 248–253.

Lestari, K., Hwang, J. K., Kariadi, S. H., Wijaya, A., Ahmad, T., Subarnas, A., Supriyatna, & Muchtaridi, M. (2012). Screening for PPAR gamma agonist from Myristica fragrans Houtt seeds for the treatment of type 2 diabetes by in vitro and in vivo. *Medical and Health Science Journal*, *12*, 7–15.

Lin, J., Cao, C., Tao, C., Ye, R., Dong, M., Zheng, Q., Wang, C., Jiang, X., Qin, G., Yan, C., Li, K., Speakman, J. R., Wang, Y., Jin, W., & Zhao, J. (2017). Cold adaptation in pigs depends on UCP3 in beige adipocytes. *Journal of Molecular Cell Biology*, *9*(5), 364–375. https://doi.org/10.1093/jmcb/mjx018 https://doi.org/10.1093/jmcb/mjx018

Nakatani, T., Tsuboyama-Kasaoka, N., Takahashi, M., Miura, S., & Ezaki, O. (2002). Mechanism for peroxisome proliferator activated receptor-alpha activator induced up-regulation of UCP2 mRNA in rodent hepatocytes. *Journal of Biological Chemistry*, *277*(11), 9562–9569.

Nissen, S., & Wolski, K. (2010). Rosiglitazone revisited: An updated meta-analysis of risk for myocardial infarction and cardiovascular mortality. *Archives of Internal Medicine*, *170*(14), 1191–1201. https://doi.org/10.1001/archinternmed.2010.207

Ohno, H., Shinoda, K., Spiegelman, B. M., & Kajimura, S. (2012). PPAR gamma agonists induce a white-to-brown fat conversion through stabilization of PRDM16 protein. *Cell Metabolism*, *15*(3), 395–404.

Okla, M., Kim, J., Koehler, K., & Chung, S. (2017). Dietary factors promoting brown and beige fat development and thermogenesis. *Advances in Nutrition: An International Review Journal*, *8*(3), 473–483. https://doi.org/10.3945/an.116.014332

Periasamy, M., Herrera, J. L., & Reis, F. C. G. (2017). Skeletal muscle thermogenesis and its role in whole body energy metabolism. *Diabetes & Metabolism Journal*, *41*(5), 327–https://doi.org/10.4093/dmj.2017.41.5.327 https://doi.org/10.4093/dmj.2017.41.5.327

Petrovic, N., Walden, T. B., Shabalina, I. G., Timmons, J. A., Cannon, B., & Nedergaard, J. (2010). Chronic peroxisome proliferator-activated receptor gamma (PPAR gamma) activation of epididymally derived white adipocyte cultures reveals a population of thermogenically competent, UCP1-containing adipocytes molecularly distinct from classic brown adipocy. *Journal of Biological Chemistry*, *285*(10), 7153–7164.

Pratiwi, Y. S., Lesmana, R., Goenawan, H., Sylviana, N., Setiawan, I., Tarawan, V. M., Lestari, K., Abdulah, R., Dwipa, L., Purba, A., & Supratman, U. (2018). Nutmeg extract increases skeletal muscle mass in aging rats partly via IGF1-AKT-mTOR pathway and inhibition of autophagy. *Evidence-Based Complementary and Alternative Medicine*, *2018*(2810840), 1–9. https://doi.org/10.1155/2018/2810840 https://doi.org/10.1155/2018/2810840

Ravussin, Y., Xiao, C., Gavrilova, O., & Reitman, M. L. (2014). Effect of intermittent cold exposure on brown fat activation, obesity, and energy homeostasis in mice. *PLoS One*, *9*(1), 1–9. https://doi.org/10.1371/journal.pone.0085876

Richard, J. E., López-Ferreras, L., Chanclón, B., Eerola, K., Micallef, P., Skibicka, K. P., & Wernstedt Asterholm, I. (2017). CNS β3-adrenergic receptor activation regulates feeding behavior, white fat browning, and body weight. *The American Journal of Physiology Metabolism*, *313*(3), E344–E358.

Rui, L. (2017). Brown and beige adipose tissues in health and disease. *Comprehensive Physiology*, *7*(4), 1281–1306.

Schweiger, M., Eichmann, T. O., Taschler, U., Zimmermann, R., Zechner, R., & Lass, A. (2014). Measurement of lipolysis. *Methods in Enzymology*, *538*, 171–193.

Seale, P., Conroe, H. M., Estall, J., Kajimura, S., Frontini, A., Ishibashi, J., Cohen, P., Cinti, S., & Spiegelman, B. M. (2011). Prdm16 determines the thermogenic program of subcutaneous white adipose tisue in mice. *Journal of Clinical Investigation*, *121*(1), 96–105.

Siannoto, M., Nugraha, G. I., Lesmana, R., Goenawan, H., Tarawan, V. M., & Khairani, A. F. (2020). The roles of nutraceuticals as inductors of WAT browning process. *Current Nutrition & Food Science*, *16*, 1–15.

Sluse, F. E. (2012). Uncoupling proteins: Molecular, functional, regulatory, physiological and pathological aspects. In R. Scatena (Ed.), *Advances in mitochondrial medicine*, *advances in experimental medicine and biology* (pp. 137–156). Springer Science + Business Media B.

Solas, M., Milagro, F. I., Martínez-urbistondo, D., Ramirez, M. J., & Martínez, J. A. (2016). Precision obesity treatments including pharmacogenetic and nutrigenetic approaches. *Trends in Pharmacological Sciences*, *37*(7), 575–593. https://doi.org/10.1016/j.tips.2016.04.008

Steinbeck, K. S., Lister, N. B., Gow, M. L., & Baur, L. A. (2018). Treatment of adolescent obesity. *Nature Reviews Endocrinology*, *14*(6), 331–344. https://doi.org/10.1038/s41574-018-0002-8

Veronica, F., Lubis, L., S, A., Fitri, L. L., Rizal, A., P, A., Gunawan, H., Lesmana, R., Dandan, K. L., & Supratman, U. (2018). A preliminary study of the effect of PPAR-γ agonist from Myristica fragrans houtt seed extract on the biogenesis of rat infant's brain mitochondria and D1 dopamine receptor. *Bali Medical Journal*, *7*(3), 574–577. https://doi.org/10.15562/bmj.v7i3.1027

Villarroya, F., Iglesias, R., & Giralt, M. (2007). PPARs in the control of uncoupling proteins gene expression. *PPAR Research*, *2007*, 1–12. https://doi.org/10.1155/2007/74364

Marroquin, F. E. M., & Willoughby, D. S. (2015). Factors regulating uncoupling protein-3 (UCP-3) expression in skeletal muscle. *Journal of Nutritional Health & Food Engineering*, *3*(1), 285–291. https://doi.org/10.15406/jnhfe.2015.03.00101

World Health Organization. (2018). *Obesity and overweight* [Internet]. Available from: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight

Wu, J., Cohen, P., & Spiegelman, B. M. (2013). Adaptive thermogenesis in adipocytes: Is beige the new brown? *Genes & Development*, *27*(3), 234–250. https://doi.org/10.1101/gad.211649.112

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Lesmana R, Siannoto M, Nugraha GI, et al. Nutmeg extract potentially alters characteristics of white adipose tissue in rats. *Vet Med Sci*. 2021;7:512–520. https://doi.org/10.1002/vms3.383