

Anti-Obesity Effect of Simvastatin and/or Omega-3 on Obese Male Wistar Rats

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Abstract

Strategies to reduce obesity have become main priority for many health institution and health staff around the world, as the prevalence of obesity has risen and exacerbated in most of the world mainly because of the modern life style which tend to be more sedentary with an increase eating unhealthy fast western food. Many years ago, the lipid-lowering drug simvastatin; and omega-3 were considered as a traditional lipid-lowering drug that have been well-documented to possess anti-inflammatory, cardioprotective and triglyceride-lowering properties; and their co-administration may demonstrate a complementary effect in lowering patients' triglycerides and total cholesterol to treat atherosclerosis. Many previous studies have been found other beneficial effects for simvastatin, and omega-3; since, simvastatin can be used for the treatment of Alzheimer's disease; and for prevention of prostate cancer; while omega 3 can reduce the risk of sudden cardiac death in addition for preventing obesity that has been documented by recent studies. But, the effect of simvastatin alone or its combination with omega-3 as potential anti-obesity therapy and /or protection against obesity is not yet known through their effects on thermogenic factors. The purpose of the current study is to evaluate the effect of simvastatin and omega-3 on thermogenic genes including (UCP1) using quantitative real time PCR, and the expression of uncoupling protein 1 (UCP1) protein was detected in iBAT and iWAT adipocyte by immunohistochemistry. One hundred and twenty (120) male Wistar rats at age five to six week, and weighing 100-150g were allocated into five groups, Group I is the obese rat that receive high fat diet only. Group II rat receive simvastatin 9 mg/kg/day; Group III rats treated with 18mg/kg/day simvastatin, Group IV rat receive omega-3 (1ml/day); Group V rat receive mixed treatment (i.e., simvastatin 9mg +omega -3 (1ml/day). Treatments were given along the eight weeks. Three rats from each group were weekly-authenticated along the 60 days interscapular brown adipose tissue (iBAT) and inguinal white adipose tissues (iWAT) were obtained. Simvastatin and omega-3 have an obvious activation of UCP1 genes; this reflects an increase in thermogenic process in adipose tissue in obese high fat diet rats and their combination exert a synergistic increase in the thermogenic mechanism when compared to simvastatin 9mg/ kg /day alone. Our results give a hope for the utilization of simvastatin either alone or in combination with omega-3 as anti-obesity therapy; through their enhancement of thermogenic in white and brown adipose tissues with a consequent weight loss.

Keywords: Obesity, High-fat diet, Simvastatin, Omega-3, brown adipocyte, Beige adipocytes, Thermogenesis, UCPI.

الآثار المضادة للسمنة للسيمفاستاتين والأوميغا - ٣ وتركيبها على فئران ويستار الذكور ذات الطراز السمين

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الخلاصة

أصبحت استراتيجيات الحد من السمنة أولوية رئيسية بالنسبة للعديد من المؤسسات الصحية والموظفين الصحيين في جميع أنحاء العالم، حيث أن انتشار السمنة قد ارتفع وتفاقم في معظم أنحاء العالم، ويرجع ذلك أساساً إلى أسلوب الحياة الحديث الذي يميل إلى أن يكون أكثر استقراراً مع زيادة تناول الأغذية الغربية السريعة غير الصحية. قبل سنوات عديدة، كان الدواء المخفض للدهون سيمفاستاتين وأوميغا-٣ عقارات تقليدية لخفض الدهون تم توثيقها توثيقاً جيداً لامتلاكهم خصائص مضادة للالتهابات وحماية القلب وخفض الدهون ثلاثية الجليسيريدات؛ وقد تظهر إدارتهم المشتركة تأثيراً تكميلياً في خفض نسبة الدهون ثلاثية الجليسيريدات للمرضى وإجمالي نسبة الكوليسترول لعلاج التهاب الأوعية الدموية. كذلك وجدت العديد من الدراسات السابقة تأثيرات مفيدة أخرى للسيمفاستاتين، والأوميغا-٣. إضافة إلى ذلك يمكن استخدام السيمفاستاتين لعلاج مرض الزهايمر؛ والوقاية من سرطان البروستاتا؛ في حين أن omega 3 يمكن أن يقلل من خطر الوفاة القلبية المفاجئة بالإضافة إلى الوقاية من السمنة التي وثقتها الدراسات الحديثة. ولكن تأثير السيمفاستاتين وحده أو توليفه مع أوميغا-٣ كعلاج محتمل ضد السمنة و/أو الحماية من السمنة غير معروف حتى الآن من خلال آثارها على العوامل الحرارية. هذه الدراسة تهدف إلى تقييم تأثير السيمفاستاتين والأوميغا ٣ على الجينات الحرارية بواسطة الكيمياء المناعية per بما في ذلك التعبير البروتيني ل ucp1 في ال الشحوم البيضاء والشحوم البنية. مائة وعشرين (١٢٠) ذكر (تم تخصيص خمسة-سبعة أسابيع من العمر ووزنها ١٠٠-١٥٠ غ) في خمس مجموعات: معالجة بجرعتين مختلفتين من السيمفاستاتين والأوميغا-٣. أظهرت أن السيمفاستاتين والأوميغا-٣ لديهما تنشيط واضح من UCP1 gene والعلاج المختلط، بالإضافة إلى مجموعة غذائية عالية الدهون التي تعتبر مجموعة مراقبة. تم إعطاء العلاج لمدة ثمانية أسابيع. تم التضحية أسبوعياً بثلاثة فئران من كل مجموعة على مدى ٦٠ يوماً وقد تم الحصول على الأنسجة الدهنية البنية بمنطقة بين الاكتاف والأنسجة الدهنية البيضاء بالمنطقة الأربية.

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وهذا يعكس زيادة في العملية الحرارية في الأنسجة الدهنية في فئران الحمية ذات الدهون العالية السمنة وتركيبها يؤدي إلى زيادة تآزرية في الآلية الحرارية عند مقارنتها بالسيمفاستاتين 9mg/كجم/يوم فقط. هذه الدراسة تعطي الأمل في استخدام السيمفاستاتين إما بمفرده أو بالاقتران مع أوميغا-3 كعلاج مضاد للسمنة؛ من خلال تعزيزها الحراري في الأنسجة الدهنية البيضاء والبنية مع ما يترتب على ذلك من فقدان الوزن. الكلمات المفتاحية: السمنة، والحمية عالية الدهون، والسيمفاستاتين، والأوميغا-3، والأديوكايت البني، والأديوكايت الحبيبية، وتوليد الحرارة، UCPI

Introduction

Obesity is a complex, multifactorial, disease, affecting, over a third of the all-world's population⁽¹⁾. It is typically defined as an increased energy intake combined with less energy expenditure⁽²⁾. The change in life style from highly active, hard- working jobs to sedentary life results in imbalance in weight gain⁽³⁾ and as a result obesity occurs; with increase in the risk of morbidity due to chronic diseases mainly type 2 diabetes and cardiovascular disease^(4, 5), with increasing in mortality scales⁽⁶⁾. Adipose tissue has emerged as a dynamic organ and plays an important role in the pathogenesis of obesity and its associated metabolic disorders^(4, 7). It is classified into white adipose tissue (WAT) and brown adipose tissue (BAT), which are visibly distinguishable according to tissue color however they differ in shape, size, and the intracellular structure of their organelles⁽⁸⁾. The BAT is mainly proposed to maintain thermal homeostasis through dissipating the energy in the form of heat⁽⁹⁾ by specific protein located in the mitochondria known as uncoupling protein I (UCPI)⁽¹⁰⁾. Moreover, WAT can play a role in thermogenesis through its browning resulting in what is known as beige/brite cells⁽¹¹⁾, which have increased mitochondrial proteins and UCPI⁽¹²⁾. Many studies observed that activation of BAT and increasing the beige/ brite cells in WAT may exert greater metabolic benefits and are associated with improvement in many physiological parameters such as a reduction in blood glucose levels and weight reduction⁽¹³⁾, through increasing resting energy expenditure with a subsequent anti-obesity effect^(14, 15). Studies hypothesized that UCPI gene can be enhanced through activating many transcriptional complexes of thermogenic genes mainly PR domain containing 16 (PRDM16) and peroxisome proliferator-activated receptor-gamma coactivator alpha (PGC-1 α) coactivators at thermogenic and their binding with DNA. Studies hypothesized that PRDM16 might be recruited to the enhancer region of the UCPI gene through the interaction with PGC-1 α and a very large increase in the uncoupled fraction of respiration⁽¹⁶⁾. A previous study showed that simvastatin could potentially help to ameliorate metabolic abnormalities associated with a long-term olanzapine treatment partly via activating the function of BAT. These findings support a potential mechanism of simvastatin in ameliorating olanzapine-induced weight gain through the mediation of energy expenditure⁽¹⁷⁾. Omega-3 fatty acids [ω -3 long chain polyunsaturated fatty acids (ω -3 PUFAs)], composed of docosahexaenoic acid (DHA) and

icosapentaenoic acid (EPA). Animal studies suggested that increased consumption of the long-chain omega-3 can protect against the development of obesity in animals exposed to an obesogenic diet and reduce body fat when already obese^{(18) (19)}. A recent study suggested that EPA can activate thermogenic transcription factors in brown fat, namely, PRDM16, PGC1 α , and PPAR γ . This led to increased expression of UCPI in BAT which may subsequently contribute to energy expenditure and possibly reduced obesity and metabolic disorders⁽²⁰⁾. The aim of the present work is to evaluate the effect of simvastatin and omega-3 and there combination as anti-obesity drugs through their effect on the most common thermogenic gene marker "UCPI" using quantitative real time PCR in white and brown adipocyte of obese male Wistar rat model where the obesity had been induced using high fat diet.

Material and methods

Animals and experimental design

The research protocol and animal care procedures were approved by the Local Research Ethics Committee, College of Pharmacy, University of Baghdad, Iraq, and in accordance with the standard requirements for the care and the use of experimental animal reported elsewhere.

One hundred and twenty (120) male Wistar rats, age of five- to six-week-old weighing 100-150g were obtained from the local bred of the animal house, Department of Pharmacology and Toxicology, University of Baghdad and housed under light/dark cycle (12/12 hr) and controlled room temperature (24 $^{\circ}$ ±2) with standard chow and drinking water *ad libitum*. After a week of acclimatization, all animals were fed for eight weeks with a high fat diet [(HFD) (standard chow contains 30% lard)] especially prepared for this purpose⁽²¹⁾ in order to induce obesity and create **an obese rat model**. Since the standard chow consist of carbohydrate 48.8%, protein 21%, and fat 3%, calcium 0.8%, phosphorus 0.4%, fiber 5%, moisture 13%, and ash 8%⁽²²⁾. After that, when rats get obese according to their body weights and body mass index (BMI) and body parameter.

Rats were randomly-allocated into five groups (each contains twenty-four rats), four receiving test compounds and one group continues feeding with HFD without any treatment and considered as a **control (Group I)**. Twelve rats were housed /cage in order to reduce their activity inside the cage and reduce energy expenditure. Rats groups were treated as follows: **Group II**: Obese (HFD) rats administered pure simvastatin powder [Ph. Eur. Artemis Biotech (9 mg /kg /day)] via 14 inch oral

gavage needle within 2cc running water⁽¹⁷⁾; **Group III**: Obese HFD rats given double dose of pure simvastatin powder (18 mg/kg/day) via 14 inch oral gavage needle within 2cc running water⁽¹⁷⁾. **Group IV**: Obese, HFD rats, given omega-3 fatty acids (oral dose 1 ml/day) [Green Field Nutrition's, Inc. Chicago, IL.60625 U.S.A. Fish Oil =1000 mg, EPA = 180 mg, DHA =120 mg] with 14-inch oral gavage needle, and the **Group V**: Obese, HFD rats, given a combination of simvastatin (9mg/kg) and omega-3 fatty acids (1 ml/day) with 14-inch oral gavage needle^(23, 24).

In this experiment, normal diet group were excluded since the main target is to focus on the reduction in body weights in obese rats receiving test compounds in order to know if there is a hope to use such test compounds in obese human in the future clinical design. Moreover, the design of this experiment depended on two different doses of simvastatin which is the main drug target required to be studied for its anti-obesity effect; for this reason 9mg/kg /day dose were selected from previous study⁽¹⁷⁾ and duplication of this dose were taken into account in order to know if there is a difference in the onset of effect, in the potency, and in the possibility of occurring adverse effect in such high dose. While omega-3 dose which is (1ml/day) has been selected according to previous study⁽²⁴⁾ where they depend in their experiment on giving each rat 1ml of omega-3 rather than calculating the dose on kg. in order to ensure standardized dosage of eicosapentaenoic acid (EPA) 180 mg and docosahexaenoic acid (DHA)120 mg.

Three rats were sacrificed from each group [which is the minimum statistically acceptable rat group and has been performed in many previous studies to limit the variation (25-27)] (and this experiment have five group) in each week i.e., 15 rats euthanized weekly by diethyl ether (Romia pure chemistry/ Cambridge/UK). So, along the eight weeks- which is the total duration of this experiment -all the total number of rats i.e., 120 rats have been

killed and the interscapular brown adipose tissue (iBAT), and inguinal adipose tissue (iWAT) were dissected. This present study has different module than any other experiment modules since the accumulated effect of test compound from week one till week eight must be measured in order to detect the exactly week that the onset of weight reduction with the expression of thermogenic gene UCP1 has been occurred. The UCP1 thermogenic genes expression was measured using quantitative real time PCR and triplicate PCR amplification has been done to reduce bias. Moreover, uncoupling protein 1 (UCP1) protein in brown and white adipocyte was detected using immunohistochemistry.

Statistical analysis

Data were analyzed using SPSS/IBM version 24. The numeric data were expressed as mean \pm standard error of the mean (SEM). The statistical significance of each group in comparison with the obese/high fat diet control group was determined by post hoc analysis in addition to independent t- test to confirm the results; while comparison among all groups were tested by one way- ANOVA test. *P*-values less than 0.05 (*P*<0.05) were considered significant for all data presented in this study.

Results

Detection of UCP1 gene in brown adipose tissue

Results showed that a clear significant increase in (**Group II**) at fifth week (154.86 \pm 8.46, *P*<0.05), while (**Group III**) gave significant increase in UCP1 expression from the first week of therapy (57.1667 \pm 1.74, *P*<0.05) Moreover, (**Group IV**) gave significant increase in UCP-1 expression in week two (76.19 \pm 2.79, *P*<0.05), while (**Group V**) gave significant expression in UCP-1 from the first week of treatment (70.93 \pm 3.92, *P*<0.05) as shown in **Table 1**. Furthermore, there was significant increase (*P*<0.05) in UCP-1 expression among all groups from the first week of treatment by using one-way ANOVA test.

Table 1. Effects of two-different doses of simvastatin, omega-3, and combination of simvastatin and omega3 on the UCP1 gene expression level in brown adipose tissue (BAT) weekly.

	HFD obese	S 9 mg	S18 mg	omega-3	O+S
	Group I	Group II	Group III	Group IV	Group V
week_1	40.667 \pm 4.029	43.58 \pm 3.2	57.1667 \pm 1.74*	48.06 \pm 5.411	70.93 \pm 3.92*
week_2	17.69 \pm 2.98	64.86 \pm 6.11	140.74 \pm 10.207*	76.19 \pm 2.79*	201.6 \pm 26.15*
week_3	73.533 \pm 8.897	108.39 \pm 10.7	140.63 \pm 14.65*	166.24 \pm 18.409*	291.33 \pm 10.47*
week_4	92.933 \pm 10.22	127.47 \pm 11.15	222.66 \pm 16.43*	261.26 \pm 11.01*	396.23 \pm 45.48*
week_5	54.966 \pm 3.653	154.86 \pm 8.46*	326.3 \pm 12.77*	296.83 \pm 24.98*	512.11 \pm 48.79*
week_6	42.92 \pm 6.33	131.056 \pm 10.194*	302.5 \pm 22.33*	626.71 \pm 27.63*	977.03 \pm 41.4*
week_7	58.45 \pm 5.1	183.466 \pm 6.78*	585.86 \pm 30.99*	901.17 \pm 22.72*	929.66 \pm 44.78*
week_8	59.8 \pm 1.5	195.3 \pm 11.113*	885.18 \pm 31.76*	759.23 \pm 37.32*	939.93 \pm 28.39*

Values are expressed as mean \pm SEM; n=24 rats in each group; (*) refers to significant difference in groups (*P*<0.05) compared to **Group I**; time represent treatment in each week.

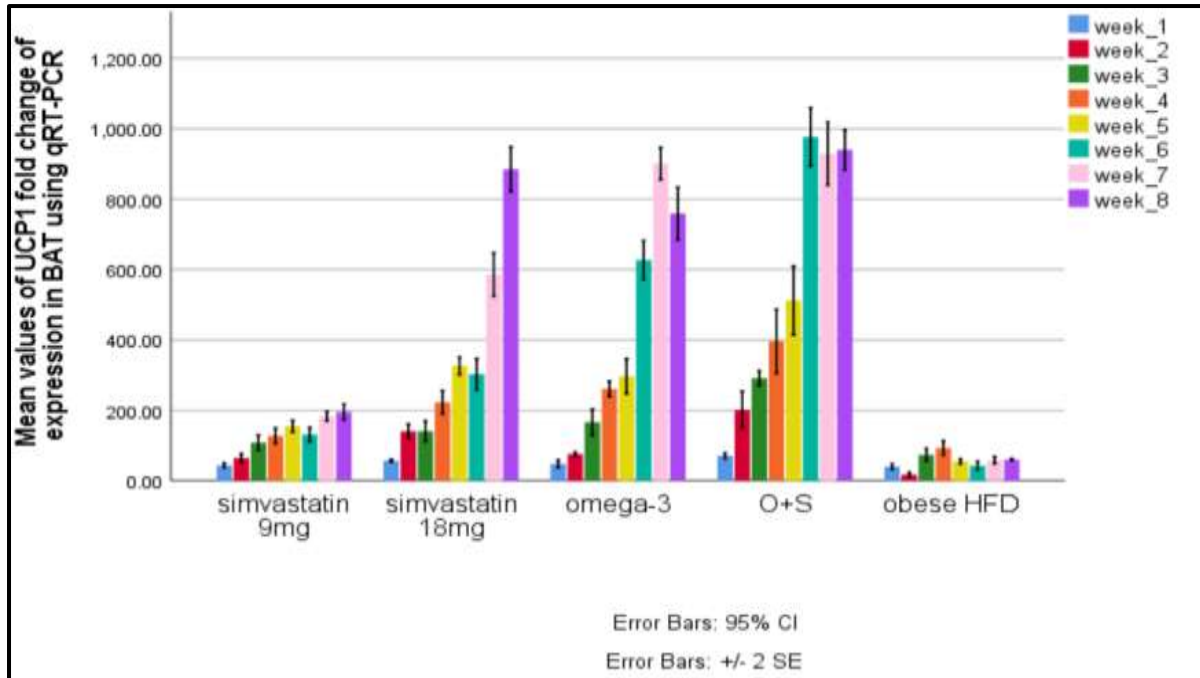


Figure 1. Effects of two-different doses of simvastatin, omega-3, and combination of simvastatin and omega3 on UCP 1 genes in brown adipose tissue (BAT).

Detection of UCPI gene in white adipose tissue (WAT)

Results showed that significant increase started from the fifth week in (Group II) when compared to obese HFD (Group I) rats (400.34 ± 9.5 , $P < 0.05$); while, obvious significant increase occurs from the fourth week in (Group III) rats (372.91 ± 32.41 , $P < 0.05$); and from the third week in (Group IV) (412.67 ± 34.42 , $P < 0.05$), and the third week in

(Group V) (412 ± 11.05 , $P > 0.05$) as shown in Table 2.

Moreover, the ANOVA test gave a significant increase among all groups from the first week of treatment which explained activation of UCPI gene in BAT from the first week of therapy. Figure 2 demonstrated effects of two-different doses of simvastatin, omega-3, and combination of simvastatin and omega3 on UCP-1 genes in white adipose tissue (WAT).

Table 2. Effects of two-different doses of simvastatin, omega-3, and combination of simvastatin and omega3 on the UCPI gene expression level in white adipose tissue (WAT) weekly.

	HFD obese Group I	S 9 mg Group II	S18 mg Group III	omega-3 Group IV	O+S Group V
week_1	180.36±16.799	64.097±7.24	107.313±2.819	97.065±4.79	191.667±6.53
week_2	213.29±22.8	162.136±2.017	131.19±10.04	195.93±2.74	191.8±11.85
week_3	285.33±16.36	270.136±8.978	229.48±15.67	412.67±34.42*	412±11.05*
week_4	135.72±13.49	176.23±8.03	372.91±32.41*	508.58±35.03*	728.62±35.5*
week_5	191.51±7.522	400.34±9.5*	445.78±50.2*	774.0667±119.6*	1557.18±91.31*
week_6	191.12±9.11	792.97±50.57*	885.014±137.93*	1622.3±139.98*	3082.5±94.01*
week_7	166.1667±25.05	811.713±91.6*9	1757.13±34.142*	1997.67±162.46*	2684.9±55.38*
week_8	173.1916.569	1221.82±123.89*	2992.8±61.28*	2826.03±99.3*	3424.28±151.041*

Values are expressed as mean ± SEM; n=24 rats in each group; (*) refers to significant difference in groups ($P < 0.05$) compared to Group I; time represent treatment in each week.

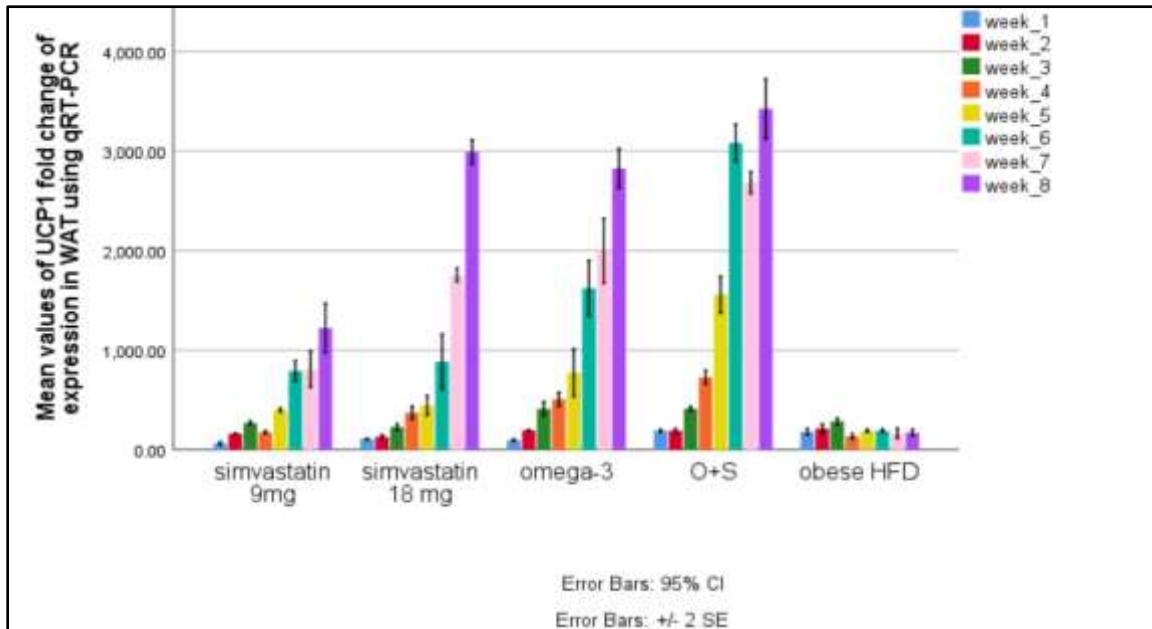


Figure 2. Effects of two-different doses of simvastatin, omega-3, and combination of simvastatin and omega3 on UCP 1 genes in white adipose tissue (WAT).

Detection of UCPI protein using immunohistochemistry

This study showed the ability of simvastatin and omega-3 to induce weight loss by activation of BAT in rodents with the recruitment of brown fat in WAT depots as shown in the image 1. (A, B, C, D, and E in white adipocyte) and (a, b, c, d, and e in brown adipocyte).

Rat weight measurements

Body weight measurements is the corner stone in this experiment, for this reason, body weight has been weekly measured in all rat groups

and results showed that there was significant decrease ($P<0.05$) in rat body weight at the fifth week in (**Group II**), and from the fourth week in (**Group III**); while in (**Group IV**); the decrease in body weight occurs at the week three of therapy; moreover, (**Group V**) gave significant decrease in body weight from first week as in **Table 3 and Figure 3, 4, 5, and 6**. While one way ANOVA test results showed a significant decrease in body weights started from the first week of therapy ($P<0.05$) among treated groups

Table 3. Values of body weight of male Wistar rats through eight weeks reduction of body weights.

	HFD obese	S 9 mg	S18 mg	omega-3	O+S
	Group I	Group II	Group III	Group IV	Group V
week_1	311±5.47	377.6±7.8	395.4±5.31	339±3.6	345.45±8.25*
week_2	348.14±5.89	367.38±7	366.2±7.7	330.5±7.8	320.8±8.35*
week_3	369.5±6.54	376±8.13	359.7±5.7	329.9±5.4*	333.8±9.5*
week_4	371.6±8.4	356±5.89	350.8±3.9*	321.2±3.9*	321.8±5.5*
week_5	372.9±6.72	337±3.46*	336.66±4.16*	316.5±5.49*	317.4±4.3*
week_6	387.3±10.1	331.5±2.93*	333.55±5.05*	315.4±6.2*	312.8±4.9*
week_7	401.33±7.62	330.8±4.85*	327.8±5.5*	315.16±7.02*	310.5±7*
week_8	417.67±4.33	255.6±8.68*	255.6±8.68*	292±4.61*	280±5.7*

Values are expressed as mean ± SEM; n=24 rats in each group; (*) refers to significant difference in groups ($P<0.05$) compared to **Group I**; time represent treatment in each week.

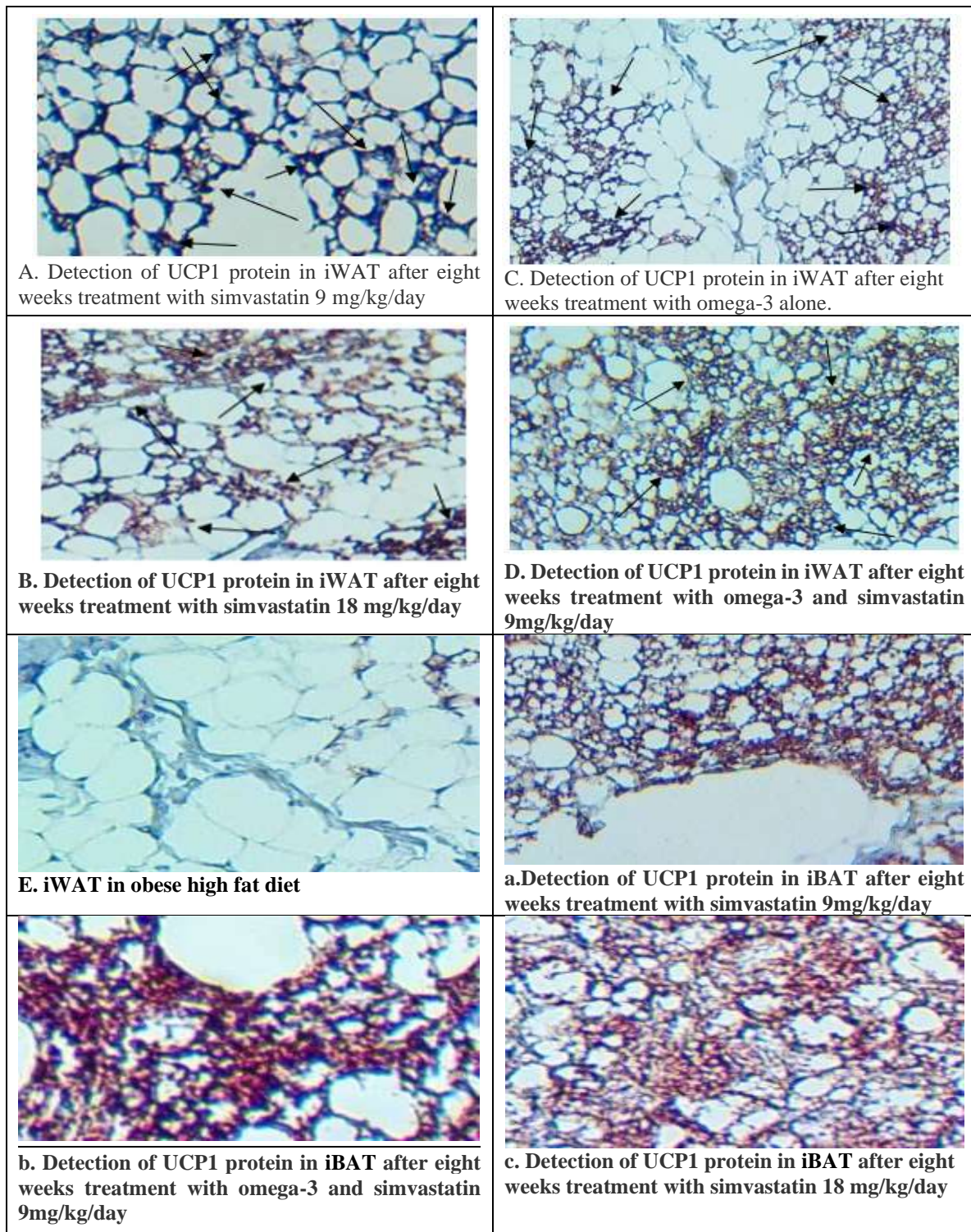
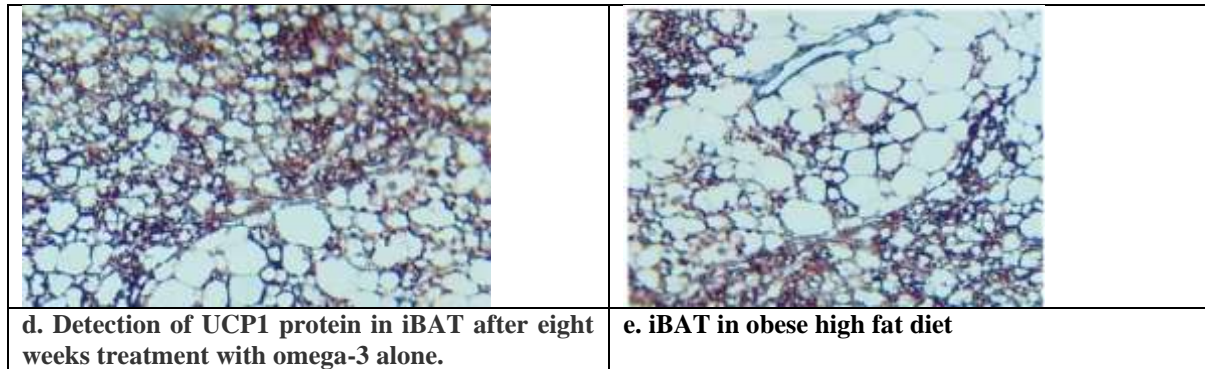


Image 1. Detection of UCP1 protein by immunohistochemistry in iWAT after eight weeks treatment(A) with simvastatin 9mg/kg/day, (B) with simvastatin 18 mg/kg/day, (C) with omega-3 alone, (D) with omega-3 and simvastatin 9mg/kg/day, compared to (E). iWAT in obese high fat diet. And detection of UCP1 protein by immunohistochemistry in iBAT after eight weeks treatment, (a) with simvastatin 9mg/kg/day, (b) with omega-3 and simvastatin 9mg/kg/day, (c) with simvastatin 18 mg/kg/day, (d) with omega-3 alone compared to (e) iBAT in obese high fat diet.



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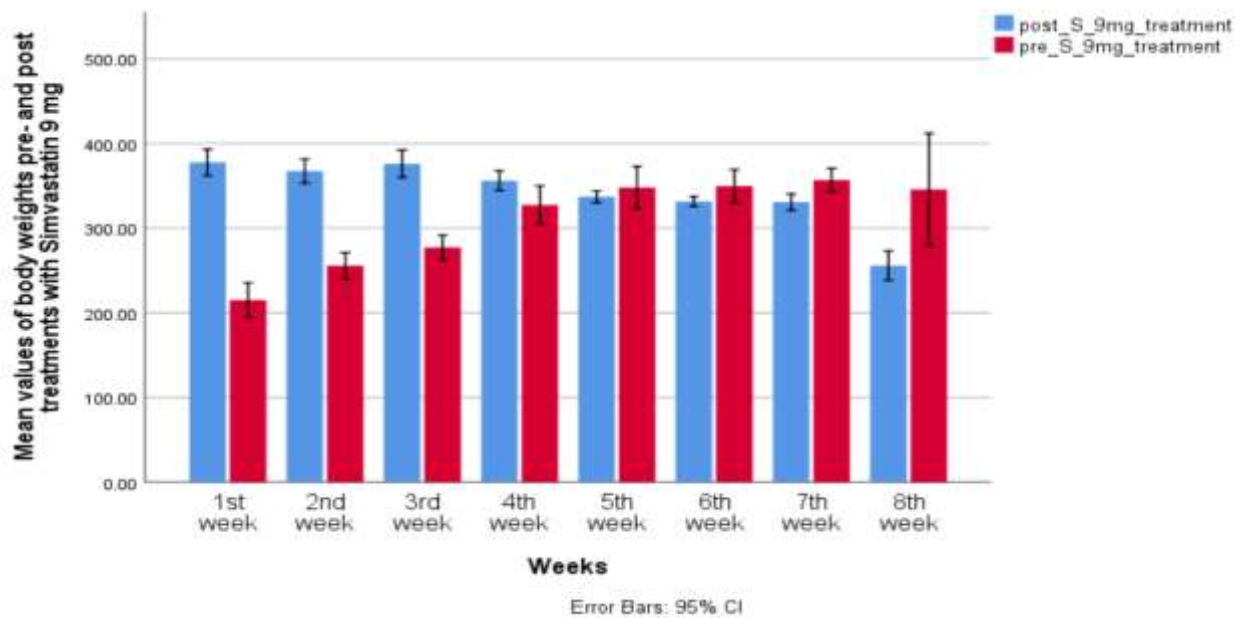


Figure 3. Demonstrate the effect of pre- and post-treatment with simvastatin 9 mg on body weights in male Wistar.

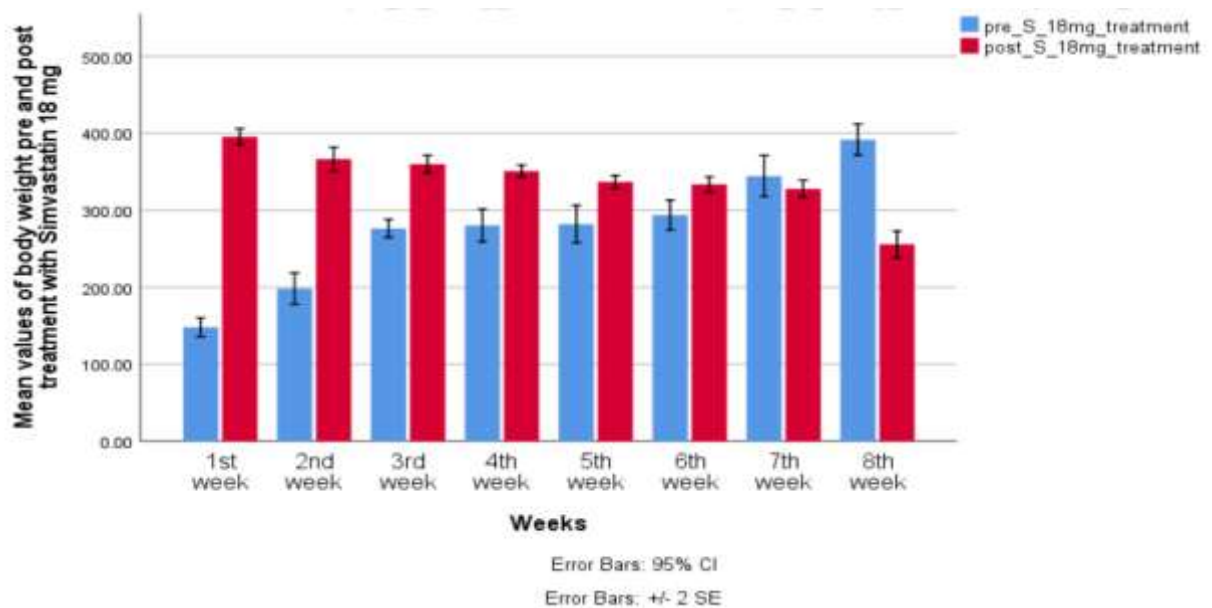


Figure 4. Demonstrate the effect of pre- and post-treatment with simvastatin 18mg on body weights in male Wistar.

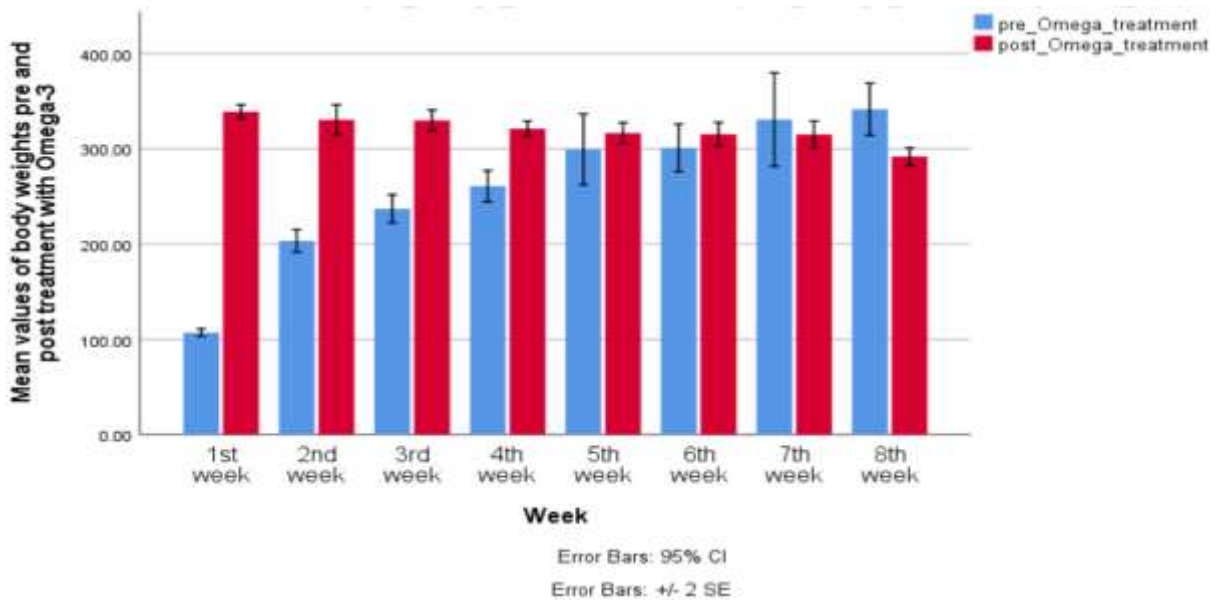


Figure 5. Demonstrate the effect of pre- and post-treatment with omega-3 (1ml/day) on body weights in male Wistar.

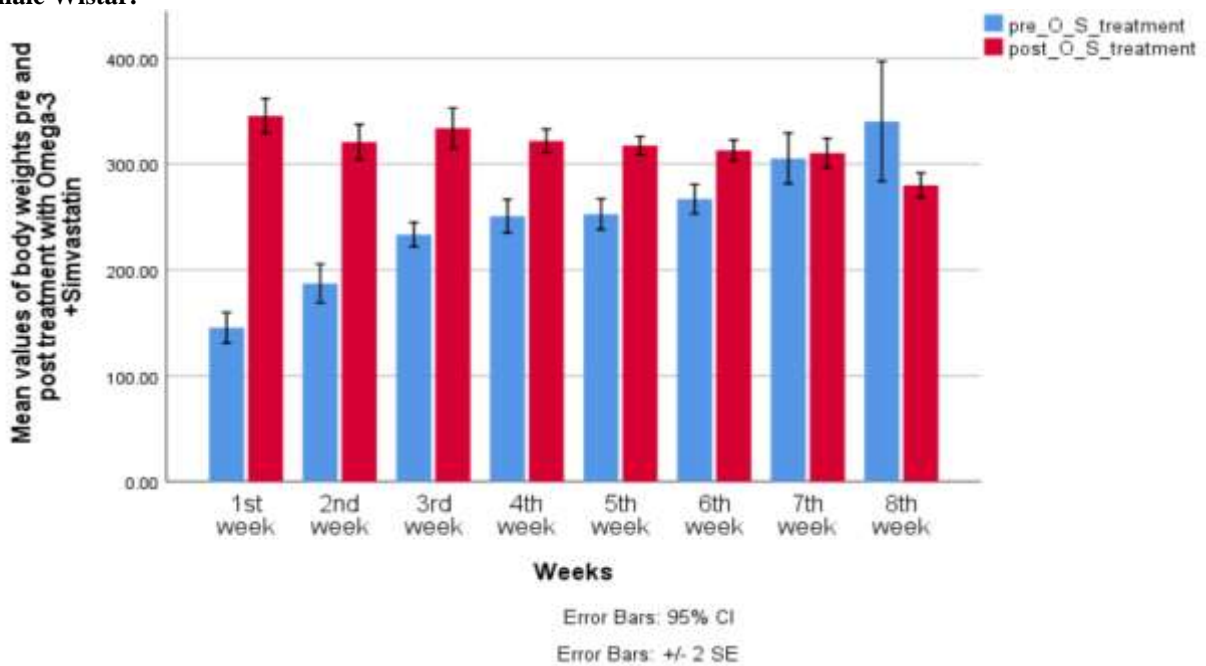


Figure 6. Demonstrate the effect of pre- and post-treatment with omega-3 and simvastatin on body weights in male Wistar rats.

Images no. 1 (A, B, C, D and E) clearly demonstrated the differences in body fat accumulation in the abdominal region of obese rats treated with two different doses of simvastatin

(Group II and III) and omega-3 (Group IV) and the co-administration of omega 3 and simvastatin (Group V) compared to HFD (Group I) rats.



A- Image represents an obese high fat diet rat without treatment (Group I)



B- Image represents an obvious weight loss after eight weeks in rats receiving 9 mg simvastatin. (Group II).



C- Image represents an obvious weight loss after eight weeks in rats receiving 18mg simvastatin. (Group III).



D- Image represents an obvious weight loss after eight weeks in rats receiving omega-3 treatment (Group IV).



E- Image represents an obvious weight loss after eight weeks in rats receiving omega-3 and simvastatin co-treatment (Group V).



Images no 1. A, B, C, D and E; represent a clear difference in body profile of male Wistar rat after eight weeks of treatment comparing to obese rat without treatment.

Discussion

This study focused on the role of simvastatin that utilized in two different doses (9mg/kg/day) and its double dose (18mg/kg/day) on the level of thermogenic genes mainly UCP1 gene in brown and white adipocyte, in an attempt to upregulation of a thermogenic program using simvastatin, omega-3 and its co-treatment; with a subsequent protection against weight gain for this aim, the induction of obesity in male rats was done with HFD (**Group I**) for eight weeks before starting the experiment. Male rat is preferred in such experimental model more than female, because induction of obesity is more easier in male, since estrogen hormones in female rats found to protect against fat adipose tissue accumulation through its action on estrogen α (ER α) and estrogen β (ER β) (28).

Studies found that, once precursor cells are induced to differentiate into brown/brite adipocytes by growth factors or hormones, a whole network of transcriptional modulators are activated to regulate the expression of specific genetic programs which are responsible for the acquisition of their unique phenotype and function (19, 20). Early studies indicated that both white and brown/brite adipocytes share a transcriptional cascade that controls the adipogenic process, including the transcriptional factors CCAAT/Enhancer Binding Protein C/EBP β , C/EBP α and PPAR γ that act sequentially to control the hundreds of genes involved in the common adipogenic program (29). However, the differentiation of precursors cells into brown/brite adipocytes requires specific transcriptional regulators that are responsible for the acquisition of their differential "thermogenic" feature including PRDM16 protein which has been revealed as a key element for the differentiation of both brown and brite adipocytes and the peroxisome proliferator activated receptor gamma co-activator 1 α (PGC1 α) (20, 30). However, according to previous study; the expected thermogenesis mechanism could be postulated as the following; PPAR γ recruits PRDM16 transcription factor to form a core transcription complex (31) and PPAR γ / PRDM16 complex recruits Pgc-1 α (32), Pgc-1 α , which considered as a transcriptional co-activator, activates cAMP dependent PKA, responsible for lipolysis and thermogenesis in BAT through β 3 receptor control under NE (33); and this leads to increased expression of UCP 1 in brown/beige adipose tissue which may subsequently contribute to energy expenditure with a possibility to reduce obesity and metabolic disorders (20, 34, 35). Brown adipose tissue (BAT) considered as an important thermogenic tissue; the thermogenic mechanism occurs when free fatty acids are burned in brown adipocytes during uncoupling respiration when uncoupling protein 1 (UCP1) are inserted in the inner mitochondrial membrane to produce the heat necessary for maintenance of the euthermic state (36).

Omega-3 fatty acids supplements provides various health benefits in a tissue-specific manner; since many studies have been shown that administration of omega-3 results in increase energy expenditure in muscle (37). Furthermore, the metabolic benefits of PUFA derived from fish oil resemble the adaptive metabolic responses upon brown/beige fat activation through activation of adaptive thermogenesis process. For this reason, n-3 PUFA intake has gained attention as a dietary regimen to promote thermogenesis (38). Therefore, omega-3 can exert dual benefits in obesity by reducing lipid accumulation in WAT with a consequent activation of thermogenesis and reducing lipogenesis in BAT (39). Omega-3 polyunsaturated fatty acids induce brown and beige adipocyte differentiation and thermogenic activation (18), and these effects require GPR120. GPR120 activation results in activation of thermogenic transcription factors in brown fat, namely, PRDM16, Pgc-1 α , and PPAR γ . This leads to increase expression of UCP 1 in brown adipose tissue with a consequence increase in energy expenditure and reduction in body weight (4, 40). This study gave a significant increase in UCP1 expression level in BAT from fifth week of simvastatin 9mg/kg/day treatment (**Group II**), while simvastatin treatment 18mg/kg/day (**Group III**) and omega-3 combined with simvastatin (9mg/kg/day) (**Group V**) gave significant increase in UCP1 expression from the first week of therapy. Moreover, treatment with omega-3 (**Group IV**) gave significant increase in UCP-1 expression from second week when compared to obese HFD (**Group I**) rats.

Results in white adipose tissue (WAT) demonstrate a significant up-regulation in UCP1 gene expression level from the fifth week after treating with simvastatin 9mg/kg/day treatment (**Group II**), and from fourth week of simvastatin at 18mg/kg/day therapy (**Group III**). However, treating with omega-3 (1ml/day) (**Group IV**) results from increase UCP1 gene expression from third week of therapy, also in case of mixed dose of simvastatin and omega-3 (**Group V**) rats where UCP1 gene expression level from increased from third week of therapy (4).

To our knowledge no study was conducted to study the effect of simvastatin, omega 3 and its co-treatment. Thus, we cannot have a chance to compare results of this study with others. Moreover, the current study opens the door for further research in this field.

Conclusion

This study gives a hope for the utilization of simvastatin and omega-3 and its combination has been shown to have the ability to turn on a thermogenic gene program in brown fat and activate a "browning" of white adipose tissues mainly UCP1. With an obvious detection of UCP1 proteins using

IHC in BAT and WAT which refer to a remarkable increase in uncoupled respiration in brown and white adipose tissue and consequently reduction in body weight.

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