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Fish Oil Supplementation Reduces Obesity Caused by a High-Fat Diet: Investigating Epigenetic Change and Genes Linked to Adipose Tissue Dysfunction in Mice

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Abstract: This research examined the effects of treating mice with fish oil (FO), specifically enhanced with eicosapentaenoic acid (EPA), on obesity brought on by a high-fat diet (HFD).Clarifying the effects of FOonepigenetic changes in white adiposetissue (WAT) and the role of adipose-derived stem cells (ASCs) was the main goal of the study. For 16 weeks, C57BL/6j mice were split into two groups: control diet and HFD.The HFD group was split into HFD and HFD + FO (treated with FO) during the course of the final eight weeks. ASCs were extracted, cultivated, and given leptin treatment, while WAT was excised in order to extract RNA and proteins.Western Blot tests, RT-PCR, and PCR-array were among the functional genomics methods used to analyse all samples. HFD-fed mice showed increased body mass, fat accumulation, and altered expression of genes linked to WAT inflammation and dysfunction.FO supplementation reduced these effects and may have a preventive effect against obesity brought on by a high-fat diet. Analysis of H3K27 showed that FO therapy partly corrected the histone alterations brought on by HFD. Leptin signalling in ASCs was further investigated in this work, which may provide a mechanism for ASC malfunction in the obesity-rich leptin milieu of WAT. All things considered, FO supplementation was effective in reducing HFD-induced obesity, affecting molecular and epigenetic pathways, and illuminating the part played by ASCs and leptin signalling in WAT dysfunction linked to obesity.

Keywords: obesity;inflammation;H3K27;n-3PUFA;WAT;leptin

1. Introduction

Inflammation and metabolic dysfunction are triggered by the high-fat diet (HFD) obesity induction paradigm, which leads to the development of obesity-related complications such insulin resistance and cardiovascular illnesses [1–3]. One prominent feature, especially when it comes to white adipose tissue (WAT), is the existence of a persistent state of flow-grade inflammation, sometimes referred to as "metaflammation," which is crucial in obesity-related health problems [3–7].In addition to identifying or directing the development of therapies targeted at particular inflammatory pathways made worse by obesity, an understanding of how inflammation is modulated during obesity can offer important insights into the underlying mechanisms of these conditions and lead to new approaches for their treatment and management. Both natural and synthetic therapies, such as those that increase insulin sensitivity and secretion while lowering glucose levels, are becoming more and more popular in the management of obesity brought on by a high-fat diet (HFD) [8,9].Similarly, it has been extensively shown that long-chain polyunsaturated fatty acids n-3 (n-3 PUFA), particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) present in fish oil (FO), improve dyslipidaemia, glucose tolerance, insulin sensitivity, and reduce adipose mass in models of obesity induced by high-fat diets [10–13].

According to our earlier research, obese rats given FO (8 weeks) supplemented with EPA in a 5:1 ratio consistently shown decreases in body weight and adipose mass, as well as improvements in adipocyte function and a decrease in the metabolic and endocrine dysfunctions linked to obesity. Additionally, FO supplementation reduced the production of pro-inflammatory cytokines, indicating that it may help prevent obesity-related endocrine diseases [14–17]. Additionally, it is known to pigenetically regulate the inflammation pathway in WAT cells.

It is generally recognised that constant remodelling is necessary for WAT to work properly in order to accommodate its quick and dynamic capacity to expand or shrink, modifying lipid reserves. Adipocyte differentiation and WAT plasticity are involved in this process, which guarantees the delivery of nutrients and oxygen to WAT as well as the proper synthesis of adipokines [18,19].ASCs, or adipose tissue-derived mesenchymal stem cells, are common in WAT and undergo adipogenesis via epigenetic modifications, namely involving the deposition of histone 3 lysine 27 (H3K27) marks [19–22]. The H3K27me3 demethylases KDM6A and KDM6B have been found to counteract the effects of EZH2-induced trimethylation of H3K27 (H3K27me3), which has been shown to promote adipogenesis by up-regulating PPARγ and suppressing Runx2 (16). On the other hand, acetylation (H3K27ac) via CREBBP and EP300 (Cbp/p300) stimulates transcription and has been shown to interact with PPARγ to induce adipogenesis [25].In addition to aiding in the development of healthy adipose tissue, the function of H3K27 modifiers in promoting adipogenesis also protects against the negative consequences of hypertrophic obesity by preserving metabolic balance and lowering the risk of chronic inflammation and its related complications [21, 26].Nevertheless, the effect of H3K27marks on WAT in the context of chronic The role of inflammation in obesity is still poorly understood.The function of H3K27 in controlling inflammatory responses in fibroblasts and monocytic cells has been clarified by recent research [27, 28].For example, it has been shown that IFN-γ and LPS work together to acetylate H3K27 and so increase the production of CCL2 in monocytic cells [27]. Repressive H3K27 trimethylation has been linked to hyperinflammation in fibroblasts, according to another research [28]. These results point to possible new therapeutic intervention targets.

In any event, the dynamic character of histone modifications—like H3K27ac and H3K27me3—is essential for controlling how genes are expressed in response to different physiological and environmental stimuli. One particularly active chromatin alteration that sticks out is histone

acetylation.The availability of acetyl-CoA has a significant impact on it [29–31], and adding acetyl groups to lysine residues (like H3K27ac) creates a more open chromatin shape that is favourable for gene transcription [32].The enzyme ATP Citratelyase (ACL) catalyses the conversion of citrate and coenzyme A (CoA) into acetyl-CoA and oxaloacetate.Thus, this enzyme connects metabolism to protein acetylation processes by playing a vital role in producing acetyl-CoA, a crucial precursor in many metabolic pathways, as well as acting as a substrate for histone acetyltransferases [33]. Carrer and associates showed that consuming an HFD for four weeks, which is known to reduce ACL [34–36], led to lower levels of acyl-CoA throughout the whole WAT [34].Additionally, it has been shown that ACL influences gene regulation and controls histone acetylation levels [31,37,38].It is yet unknown how ACL expression is regulated in ASCs, especially when obesity is involved. Remarkably, it has been shown that leptin signalling lowers ACL expression in hepatocytes and adipocytes [35].But can leptin, which is widely expressed in the WAT of obese animals, operate paracrinally on ASCs to affect ACL expression, which may then depigenetically It is still unknown whether they mediate differentiation and/or inflammation in these cells to affect WAT dysfunction.

Few studies have examined the relationship between the expression of the H3K27 modifiers and/or ACL and the increased products of chronic low-grade inflammation in obese patients.Likewise, nothing is known about how HFD, with or without FO supplementation, affects H3K27ac and H3K27me3 levels in WAT. The research found that the most significantly impacted signalling system in our HFD-induced obesity mouse model was the inflammatory route driven by chemokine and cytokine signalling.Importantly, FO treatment modulated this pathway.We want to determine if this regulation includes leptin and ACL involvement, as well as epigenetic alterations of histone 3lysine 27 (H3K27). We used a variety of functional genomics techniques, such as Western Blot analyses, PCR-array, and RT-PCR, in a mouse obesity model to answer this issue.

2. Results

ObesityModelCharacterization

At the conclusion of the 12-week experimental regimen for obesity induction, obesity parameters were evaluated.As anticipated, after receiving the HFD, there was a rise in fat consumption (~5×, Figure 1A) but a reduction in food and calorie intake.A statistical difference in body mass between the control group and the HFD-receiving animal groups became apparent in the third week.Over the course of the experiment, this disparity grew, and by the 12th week of HFD administration, the animals receiving it had gained around 3.5×body mass in comparison to those getting the CO diet (Fig. 1B).

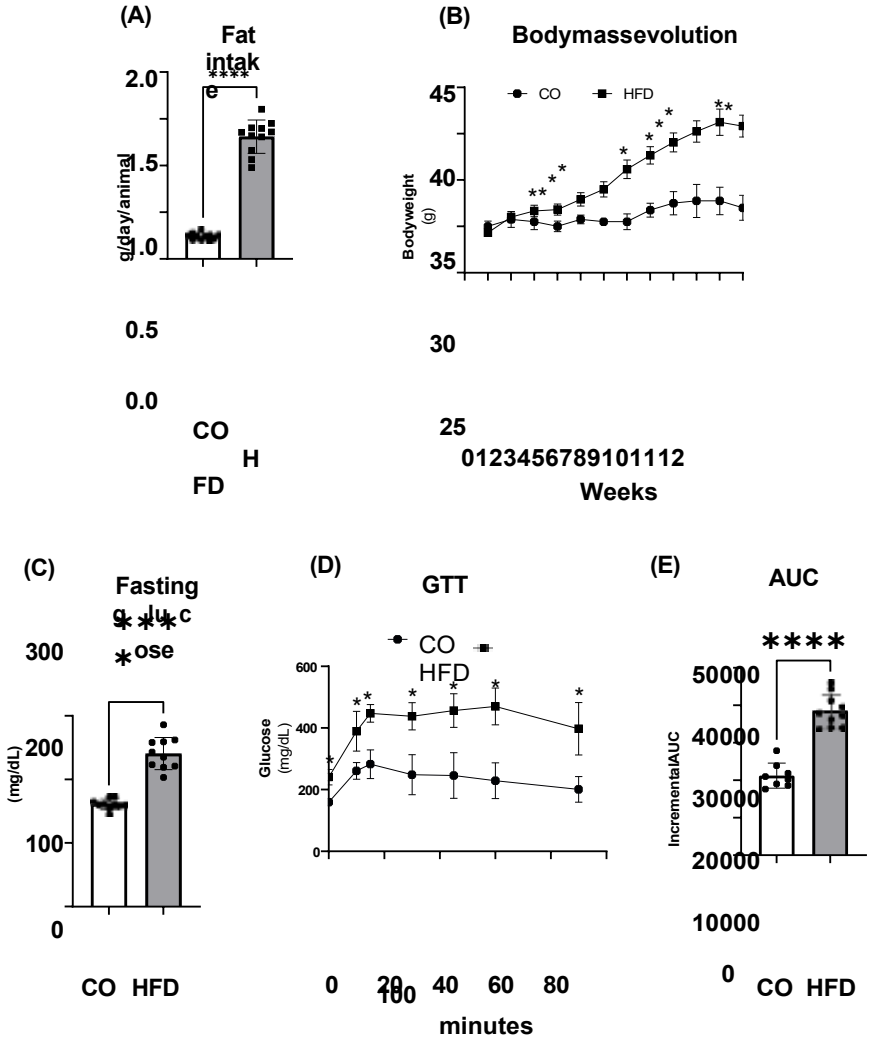


Figure 1. Obesity Model Characterization. (**A**) Caloric (kcal/day/animal), Food and Fat (g/day/animal)intake,(**B**)Bodymassevolution,(**C**)Fastingglucose,(**D**)GlucosetolerancetestorGTT,and (**E**)Incrementalareaundertheglycemiccurveincontrol(CO)andobeseanimalsinducedbyahigh

fat diet (HFD) for 12 weeks. In (**A**,**B**), the measurements were performed weekly throughout the experimentalprotocol.In(**C**–**E**),theglycemiccurveorglucoseconcentrationversustimewascalcu- lated after glucose administration (2 g/Kg b.w.). Data were analyzed using Student's *t*-test, and show mean ± SEM ($n = 12$). * $p < 0.05$ or **** $p < 0.0001$ versus control.

Figure 1C–E also illustrates values related to fasting blood glucose and the glucose tolerancetest(GTT).ComparedtotheCOdiet,micefedwiththeHFDshowedanapprox- imately50%increaseinfastingbloodglucose(Figure1C)andglucoseintolerance(Figure 1D), with anexpressive (82%) increase in the area under the curve (Figure 1E). Thus, our diet-inducedobesitymodelwascharacterized,confirmingourpreviousresults[15,17,39]. WesubsequentlysupplementedtheanimalswithFO(HFD+FOgroup)inthelast8weeks of the 16-week experimental protocol with the HFD.

WATdepotsExtractedfromMiceTreatedwithCO,HFD,andHFD+FODiets:Depot Mass and Gene Expression by PCR Array

Asexpected[15,17],miceconsumingtheHFDpresentedasignificantincreaseof50% in body mass when compared to the CO group, and the HFD + FO group was still 40% higherinrelationtotheCOgroup,butpresentedasignificantreductionof~30%(p<0.05) comparedtotheHFDgroup(Figure2A).Moreover,animalsintheHFDgroupexhibited asignificantincreaseinthemassofthevisceralepididymal(Epi)fatdepot(by~3×),while treatment with FO completely prevented this increase in fat mass (Figure 2B). However, nosignificantdifferencewasobservedbetweentheHFandHFD+FOgroupsinthemass ofthevisceralretroperitoneal(Rp)(Figure2C)orsubcutaneousinguinal(Ing)(Figure2D) fatdepots. The complete metabolic characterization of FOtreatment in this model, along withmeasurementsofothertissueweightssuchasthe liver andinterscapularbrownad- iposetissue,hasbeenpreviouslydemonstratedandreportedinstudiesconductedbyour group [14–17].

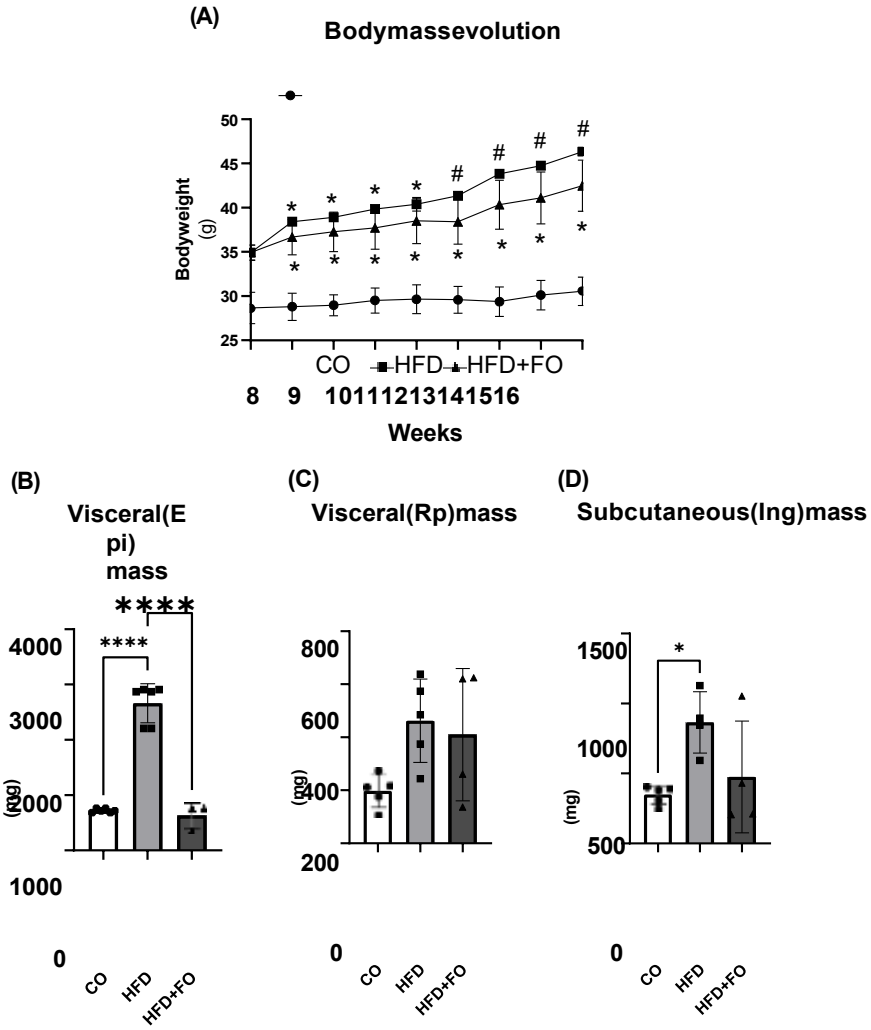


Figure2. Bodymassevolution(**A**),depotmassofvisceralepididymal(Epi)(**B**),retroperitoneal(Rp) (**C**), and subcutaneous inguinal (Ing) (**D**) adipose tissues in milligrams (mg), after 16 weeks of experimentaldietsandfishoil(FO)supplementation.Intheinitial8weeks,theanimalsweresubmit- tedtoeitheracontrol(CO)orhigh-fatdiet(HFD).Duringthelast8weeksoftheexperimentalpro- tocol, the diets were continued, and the animals underwentgavage (CO and HFDgroups received water,while the HFD+FO groupreceived FO) three times aweek. Data were analyzed using one-wayAnalysisofVariance(ANOVA)followedbyTukey'spost-test,andshowmean±SEM(n=6).* p <0.05 vs.CO, $^{\#}p$<0.05 vs. CO andHFD+FO, ****p< 0.0001.

Ananalysis of EpiWATdepots(wholetissues) wasconductedtoexploretheirgene expression profiles. To do so, we designed a customized panel of 84 essential genes for adiposetissueusingaPCRarraygeneexpressionassay.Followingthevalidationofreactionsviaarraycontrolplates,anin-depthcomparativeanalysiswasperformedonthetis- sue Ct values to thoroughly examine the gene expression changes induced by the HFD, associated (or not) withFO supplementation. Initially, our emphasiswasonscrutinizing genes with differential expression, both up- or down-regulated, attributed to the HFD when compared to the CO diet. These findings, contrasting obese animals with controls, are comprehensively outlined in Table 1.

Table1. Listofgenesthatwereup-regulatedanddown-regulated,comparingtheobesitygroupto the control group: HFD vs. CO.

Gene	RefSeqNumber	Fold Regulation	p-Value	PathwayRelated
Up-regulated				
Lep	NM_008493	25.48	0.046332	Adipokines
Ncor2	NM_001253904	3.08	ns	Anti-Browning
Dio2	NM_010050	3.98	0.000554	Pro-Browning,fattyacidthermogenesis,and oxidation
Elovl3	NM_007703	2.63	ns	Pro-Browning,fattyacidthermogenesis,and oxidation
Ccl2	NM_011333	5.07	0.009529	Cytokines,growthfactors,andsignaltransduction
Il10	NM_010548	2.08	ns	Cytokines,growthfactors,andsignaltransduction
Tgfb1	NM_011577	3.06	ns	Cytokines,growthfactors,andsignaltransduction
Tnf	NM_013693	10.65	0.009005	Cytokines,growthfactors,andsignaltransduction
Nfkb1	NM_008689	2.52	ns	Cytokines,growthfactors,andsignaltransduction
Cd68	NM_009853	9.16	0.000279	Cytokines,growthfactors,andsignaltransduction
Down-regulated				
Adipoq	NM_009605	−2.82	0.010125	Adipokines
Cfd	NM_013459	−2.54	0.004805	Adipokines
Retn	NM_001204959	−3.30	0.016985	Adipokines
Acaca	NM_133360	−2.55	ns	Lipasesandlipogenicenzymes
Scd1	NM_009127	−2.90	0.042774	Lipasesandlipogenicenzymes
Lpin1	NM_001130412	−8.55	0.001238	Lipasesandlipogenicenzymes
Pck1	NM_011044	−5.98	ns	Lipasesandlipogenicenzymes
Fasn	NM_007988	−3.87	ns	Lipasesandlipogenicenzymes
Cebpa	NM_007678	−2.45	0.002073	Pro-adipogenesis
Cebpd	NM_007679	−3.64	0.041382	Pro-adipogenesis
Fabp4	NM_024406	−2.01	ns	Pro-adipogenesis
Fgf2	NM_008006	−2.37	ns	Pro-adipogenesis
Fgf10	NM_008002	−2.71	ns	Pro-adipogenesis
Jun	NM_010591	−2.05	ns	Pro-adipogenesis
Sfrp1	NM_013834	−2.98	ns	Pro-adipogenesis

Klf15	NM_023184	−4.62	ns	Pro-adipogenesis
Adrb2	NM_007420	−6.37	0.001464	Anti-adipogenesis
Dlk1	NM_001190703	−2.36	ns	Anti-adipogenesis
Foxo1	NM_019739	−2.26	ns	Anti-adipogenesis
Shh	NM_009170	−18.41	0.000001	Anti-adipogenesis
Wnt1	NM_021279	−4.60	0.001147	Anti-adipogenesis
Wnt3a	NM_009522	−11.21	0.000001	Anti-adipogenesis
Gata2	NM_008090	−2.32	ns	Anti-adipogenesis
Bmp7	NM_007557	−2.06	ns	Pro-Browning,fattyacidthermogenesis,and oxidation
Ppargc1a	NR_027710	−2.40	ns	Pro-Browning,fattyacidthermogenesis,and oxidation
Ppargc1b	NM_133249	−2.29	ns	Pro-Browning,fattyacidthermogenesis,and oxidation
Sirt3	NM_001127351	−2.34	ns	Pro-Browning,fattyacidthermogenesis,and oxidation
Tbx1	NM_011532	−11.21	0.000001	Pro-Browning,fattyacidthermogenesis,and oxidation
Ucp1	NM_009463	−5.67	ns	Pro-Browning,fattyacidthermogenesis,and oxidation
Nr1h3	NM_001177730	−2.28	0.007073	Anti-Browning
Wnt10b	NM_011718	−2.50	ns	Anti-Browning
Lepr	NM_001122899	−2.30	ns	Adipokinesreceptors
Adipor2	NM_197985	−3.03	ns	Adipokinesreceptors
Adrb1	NM_007419	−2.73	ns	Adipokinesreceptors
Ifng	NM_008337	−11.06	0.000001	Cytokines,growthfactors,andsignaltransduction
Il4	NM_021283	−2.20	ns	Cytokines,growthfactors,andsignaltransduction
Il6	NM_031168	−10.93	0.000001	Cytokines,growthfactors,andsignaltransduction
Il13	NM_008355	−11.21	0.000001	Cytokines,growthfactors,andsignaltransduction
Insr	NM_010568	−3.52	ns	Cytokines,growthfactors,andsignaltransduction
Irs1	NM_010570	−4.26	0.043055	Cytokines,growthfactors,andsignaltransduction
Irs2	NM_001081212	−4.61	0.017955	Cytokines,growthfactors,andsignaltransduction
Pik3r1	NM_001024955	−2.33	ns	Cytokines,growthfactors,andsignaltransduction
Irf4	NM_013674	−11.92	0.000978	Cytokines,growthfactors,andsignaltransduction

ns = Not significant. This indicates that, although there was a fold-regulation greater than 2 in the array,the'*p*'valuewashigherthan0.5,likelyduetoalargevariationresultingfromalownumber of biologicalreplicates.Nevertheless,thesegenesmayplayan importantrole in thepathwaysacti- vated by FO in WAT.

Amongthevariousgenesmodulated,wehighlighttheup-regulationofthegenes*Lep* (+25.48×),*Ncor2*(+3.08×),*Ccl2*(+5.07×),*Tnf*(+10.65×),*Nfkb1*(+2.52×),and*Cd68*(9.16×),trig- geredbytheHFDwhencomparedtotheCOgroup(Table1).Withtheexceptionof*Ncor2* (anti-adipogenic factor), all these genes encode cytokines (*leptin*, *Tnfa*) and macrophage chemotactic factors (*Ccl2*/*Mcp-1*) or markers of macrophages (*Cd68*) and inflammation pathways(NFkB).Therewasalsoanincreasein*Dio2*(+3.98×)and*Elovl3*(+2.63×),indicat- ing an increase in diet-induced thermogenesis.

We also observed that the HFD suppressed the expression of numerous genes, in- cluding those encoding adipokines (adiponectin and resistin), enzymes involved in lipid biosynthesis(*Acc1*,*Scd-1*,*Lipin1*,*Pck1*,and*Fas*),factorsrelatedtoadipogenesis(*Cebpa*,*Ce- bpd*,*Fabp4*/*Ap2*,*Fgf-2*,*Fgf-10*,*AP-1*/*C-Jun*,*Sfrp1*,*Klf15*,*Adrb2*,*Dlk1*/*Pref-1*,*Foxo-1*,*Shh*,*Wnt1*, *Wnt3a*,*Gata2*),proteinsassociatedwithbrowning,thermogenesis,andfattyacidoxidatio

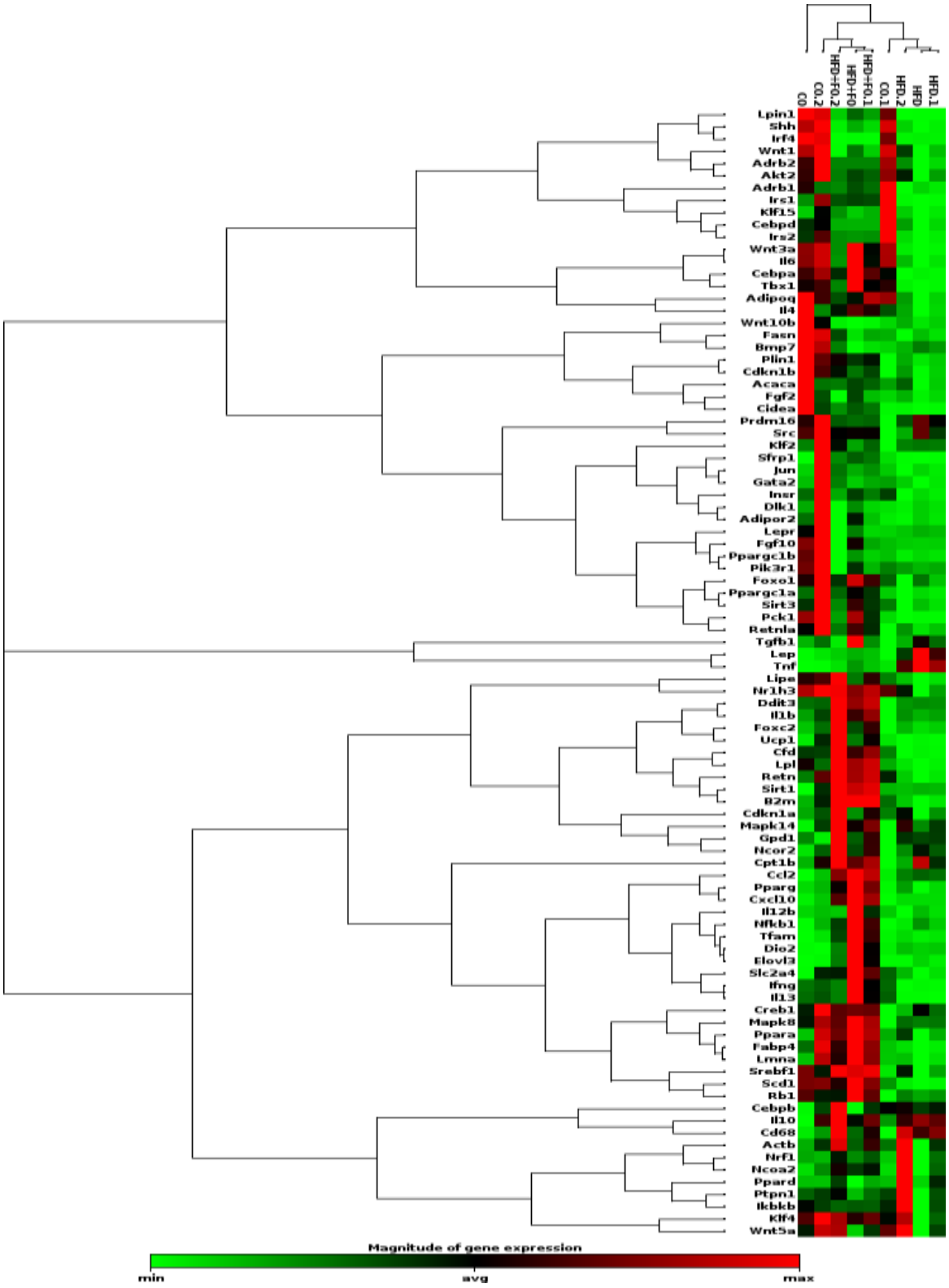


Figure3. HeatmapofgeneexpressioninEpiWATfrommicesubjectedto16weeksofexperimental diets and fish oil supplementation. Control diet (CO), high-fat diet (HFD), and high-fat diet plus fish oil (HFD + FO). The gene expression level is indicated using a color scale, where red indicates higher expression and green indicates lower expression.

ExpressionofH3K27Modifiers,H3k27ac,H3k27met3,andAcly/ACLinthevisceralEpi WAT from Mice

Regarding possible alterations in epigenetic marks, we initially assessed the expression of genes encoding enzymes responsible for acetylation (*Ep300* and *Crebbp*), as well as methylation (*Ezh2*) and demethylation (*Kdm6a* and *Kdm6b*) of H3K27 in visceral Epi WAT of control mice, obese mice (HFD), and obese mice treated with FO (HFD+FO). There was no statistically significant difference observed in the transcript levels of *Ezh2* (Figure 4B). However, the genes encoding the acetylases *Crebbp* (Figure 4C) and *Ep300* (Figure 4D), as well as the demethylase *Kdm6b* (Figure 4F), demonstrated a notable increase in expression within the HFD group compared to the control. This effect was completely reversed by FO treatment for *Crebbp* and *Ep300*, while partially reversed for *Kdm6b*. Additionally, no difference was noted between the groups in the expression of the gene encoding the en- zyme *Kdm6a* (Figure 4E).

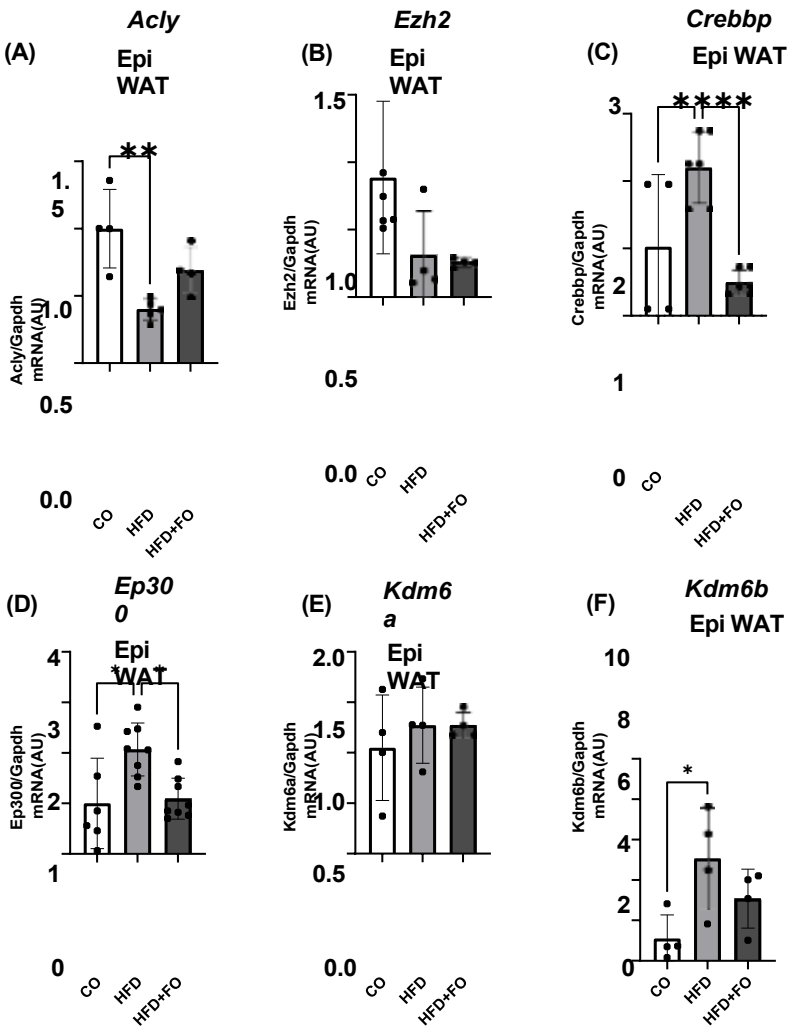


Figure 4. Gene expression of *Acly* (**A**) and genes encoding histone modifiers *Ezh2* (**B**), *Crebbp* (**C**), *Ep300* (**D**), *Kdm6a* (**E**), and *Kdm6b* (**F**), in the visceral Epi WAT from animals that received control diet (CO), high-fat diet (HFD), or HFD and fish oil (HFD + FO). Target genes were normalized by the constitutive *Gapdh*. Data were analyzed using one-way ANOVA followed by Tukey's post-test, and show mean ± SEM (n = 4–6). * $p < 0.05$ or ** $p < 0.003$ or *** $p < 0.001$.

We next investigated the expression of the *Acly* (gene) and ACL (protein), as well as histone-modifying proteins. Consistent with the decreased *Acly* expression (Figure 4A), we observed a reduction in ACL expression in the visceral Epi WAT of obese animals (Figure 5A). Corroborating these findings, we detected a decrease in the expression of H3K27ac in the group of animals with obesity induced by the HFD (Figure 5B), and once again, FO was able to completely prevent this effect. Finally, a significant decrease in the expression of the H3K27me3 protein was also observed (Figure 5C), an effect partially prevented by FO.

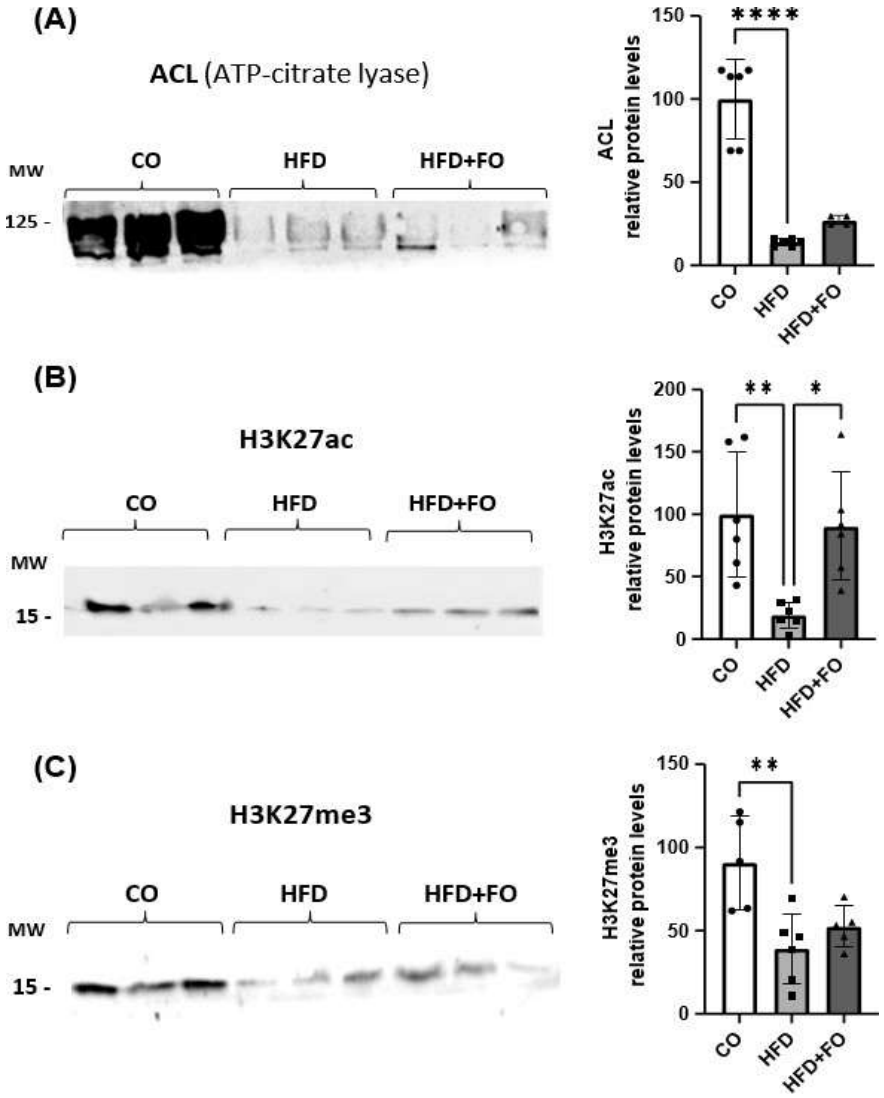


Figure5. GraphicalrepresentationoftheproteincontentofACL(**A**),H3K27ac(**B**),andH3K27me3 (**C**),invisceralEpiWATfromanimalsthatreceivedacontroldiet(CO),ahigh-fatdiet(HFD),oran HFDdietandfishoil(HFD+FO).Datawereanalyzedusingone-wayANOVAfollowedbyTukey's post-test. Values were expressed as mean ± SEM, in relation to the control and corrected by the expression of the constitutive beta-actin and total protein by Ponceau. A representative image of protein expression levels from 2 independent experiments is shown above each graph (n = 3 animals) quantified by ImageJ (Software v1.5.4e). * $p < 0.05$ or ** $p < 0.001$ or **** $p < 0.0001$.

GeneExpressionofAclyandLeptinReceptorsinASCsfromMice

InFigure6,wevalidatedtheexpressionof*Lepr1*and*Lepr2*receptorisoformsinASCs. WeobservedthatASCsextractedfromthevisceralWATshowednodifferenceinreceptor isoform expression between the CO and HFD animal groups (Figure 6A,B). Moreover, *Lepr3* isoform was not expressed in these cells. When we treated these mice ASCs with leptin (in vitro for 24 h) and examined the expression of LEP protein receptor (long iso- form, Ob-Rb, responsible for total signal transduction), we found that ASCs fully expressedthesereceptors,whoseexpressionremainedunchangedwhentheASCswereexposedtoleptin(Figure6C).Finally,weevaluatedtheexpressionofthe*Acly*geneinthese ASCs treated in vitro with leptin. Interestingly, we observed a reduction inAcly expres- sion in the HFD mice group. This finding suggests that, in a leptin-rich medium, ASCs exhibit a decrease in *Acly* expression (Figure 6D).

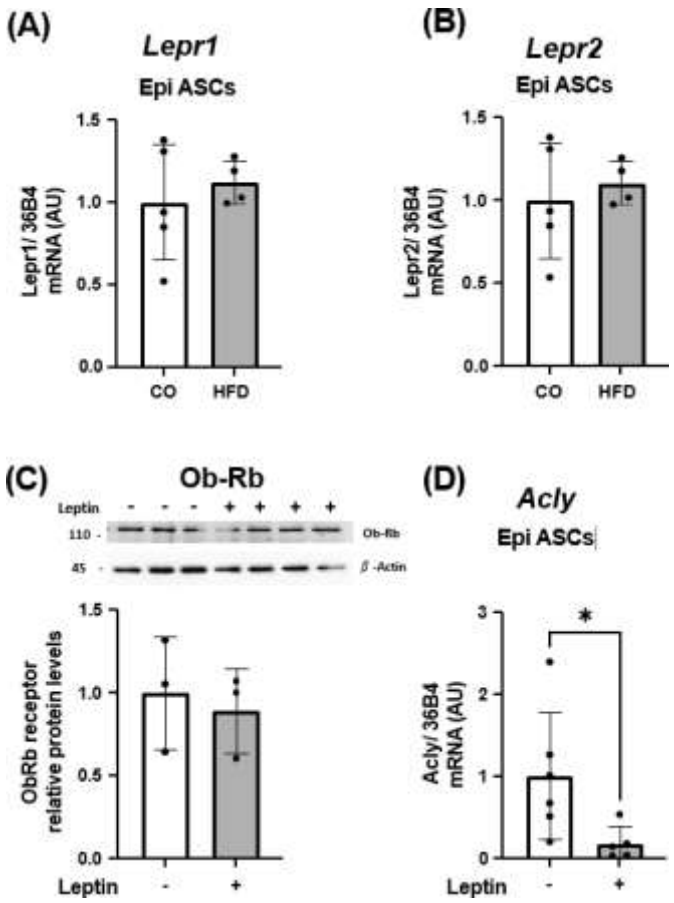


Figure 6. Gene expression of *Lepr1* (**A**) and *Lepr2* (**B**) in ASC isolated from WAT of animals that received a control diet (CO) or a high-fat diet (HFD). Total content of LEP R1 (Ob-Rb) protein (**C**) andgeneexpressionof*Acly*(**D**)inASCisolatedfromWATofHFD-inducedobeseanimals,treated invitrowith100ng/mLleptinfor24h.DatawereanalyzedusingStudent's*t*-test,andshowmean± SEM (*n* = 4–6). In (**A**–**C**), target genes were normalized by the constitutive *36B4*. In C, total content of protein was quantified by *ImageJ* and expressed in relation to the control and corrected by the expression of the constitutive beta-actin and total protein by Ponceau. A representative image of protein expression level is shown above the graphic. * $p < 0.05$.

3. Discussion

We examined whether FO therapy, which is high in EPA, shields the mouse WAT from the inflammatory pathway brought on by HFD-induced obesity.Changes in the expression of many key genes involved in WAT metabolism and cell differentiation are linked to the activation of this pathway.Furthermore, we investigated the influence of leptin (whose secretion is elevated in the WAT of obese people) on ASCs and if these effects are controlled by H3K27 changes. According to our findings, FO reduces the detrimental effects of chronic inflammation linked to obesity and influences epigenetic pathways by modifying H3K27 acetylation and ACL expression.This work further emphasises how leptin functions as a paracrine signal in addition to being a crucial endocrine signal, which has a major effect on ASCs and may eventually influence their capacity to produce fat.

The mass of visceral fat depots increased significantly in mice given the HFD for 16 weeks, and genes encoding cytokines, macrophage chemotactic factors, macrophage markers, and inflammatory pathways were all up-regulated.These results are consistent with the body of research that has been written on the many severe and harmful effects of the HFD [1].Additionally, we noticed a down-regulation in the expression of severaladipocyte receptors, pro- and anti-inflammatory cytokines, enzymes involved in lipid biosynthesis, proteins and factors linked to adipogenesis, browning, thermogenesis, and fatty acid oxidation, genes, including those encoding adiponectin, and elements of the insulin signalling system. The HFD reduced the expression of 41 genes in total.It's interesting to note that the long list of genes adversely impacted by the HFD was avoided when the animals were given omega-3 polyunsaturated fatty acids (FO). Furthermore, the FO treatment up-regulated a total of 32 genes, which clearly separated these animals from the group of obese mice and brought their results closer to that of the CO group. When combined, our findings unequivocally show that FO therapy significantly affects the chemokine and cytokine signaling-mediated inflammatory pathway in WAT taken from mice fed the HFD.

Additionally, we were especially interested in the reversal of leptin expression after FOtreatment among the genes that were changed in the array.A cytokine essential for controlling energy expenditure in adipocytes, leptin, is markedly raised in people with obesity and chronic inflammation. In this research, the HFD group's leptin expression was 25 times higher than that of the control group. Remarkably, FO therapy decreased leptin expression in the visceral Epi WAT to levels lower than those of the control group in addition to reversing this rise. It was demonstrated that leptin decreased the expression of the ACL enzyme.in both adipocytes and hepatocytes [35].Additionally, our results are supported by animal models of HFD-induced obesity, which often show decreased ACL expression in both hepatocytes and adipocytes [34–36].The overabundance of exogenous fatty acids from the food may cause the esterification of fatty acids to produce triglycerides to stay high, but physiologically, the availability of dietary fats (from HFD) results in lower demand for enovo lipogenesis and, subsequently, decreased ACL

expression/activity.

The byproduct of ACL, acetyl-CoA, serves as a substrate for histone acetyltransferases, such as CREBBP and EP300, which facilitate the acetylation of H3K27 [33]. This relationship relates the acetylation of proteins to its metabolism. Therefore, a decrease in cellular acetyl-CoA concentration may be implied by lower expression of Acly and ACL, which would restrict the amount of substrate available for histone acetylation. This behaviour is consistent with another research [34] and is likely to have happened in our model. Additionally, it has been shown that ACL affects histone acetylation, which in turn regulates gene expression [31,38,40]. Here, we found a synchronised down-regulation of Acly/ACL and a reduction in global H3K27ac levels in the visceral WAT of obese mice at the same time as the reported increase in leptin expression.Combined, our results point to a plausible link between the decrease in ACL (caused by HFD) and the subsequent decline in the availability of substrate for the acetylase enzymes Ep300 and Crebbp, whose mRNA was probably up-regulated by a regulatory feedback system and ultimately resulted in the observed decrease in H3K27ac. This decrease may have an effect on the many genes that are severely impacted by HFD, considering the critical function of H3K27 in transcriptional activation and chromatin accessibility.

Importantly, FO therapy successfully restored the significant up-regulation of leptin expression as well as the down-regulation seen in ACL and H3K27ac. Therefore, this H3K27ac reversal may help restore the regulation of genes that are often repressed in obesity, as shown in our current array research and in other investigations [31]. In addition to preventing the long list of genes that the HFD adversely affected (41 genes in total), FO treatment also up-regulated 32 genes, bringing these animals' expression profiles closer to those of the control (CO) group and clearly distinguishing them from the group of obese mice. It is important to note that FO completely reversed the decrease in H3K27ac levels, highlighting its ability to lessen the negative effects of HFD-induced obesity on gene expression patterns and indicating a protective effect against these epigenetic modifications.If the levels of histone acetylation in WAT are impacted by the consumption of an HFD, and if this effect is alleviated by FO therapy, our results may indicate that gene expression systems associated with inflammation and adipogenesis may be affected. According to our research, diets high in omega-3 polyunsaturated fats (EPA and DHA) have shown positive effects on cancer cell lines and patients, mostly by lowering inflammation via epigenetic processes (for a review, see [41,42]).This causes lobal hyperphosphorylation of histones at some locations and N-terminal regions [43].A diet high in 3PUFAs also inhibits the enzyme Acetyl-CoA Carboxylase, which increases free acetyl-CoA and provides more substrate for histone acetylation [34, 44].Furthermore, these diets alter the levels of production of certain microRNAs, most likely via altering chromatin accessibility through histone hyperacetylation [37].

It is unclear, therefore, how precisely n-3 PUFA-rich fish oil alters epigenetic markers in WAT, adipocytes, or ASCs.It is difficult to separate the distinct contributions of adipocytes and ASCs since the investigations presented here were carried out in WAT. Significant changes in WAT are associated with "unhealthy" obesity, whereas ASCs undergo adipogenesis and develop into adipocytes to sustain renewal under "healthy" settings [45].Evidence points to dysregulated adipogenesis as the cause of the disruption of WAT homeostasis in an obesogenic setting [46], with ASCs being essential for WAT remodelling during obesity [47].

The molecular processes behind obesity are poorly understood, although it is well recognised that epigenetic control plays a major role prior to the beginning of the illness. According to studies, one important and potentially regulatory event in obesity is the epigenetic malfunction of ASCs, which impairs adipocyte maturation and lowers adipogenic capacity [48,49]. Additionally, a defective pool of ASCs is present in WAT from people with obesity and/or type 2 diabetes [50–53].Obesity preconditions ASCs with a dynamic modification of DNA methylation in certain areas, which results in WAT malfunction and the development of metabolic syndromes in obese individuals. Recent research [48] suggests that DNA methylation patterns are basically retained throughout cell commitment to adipogenesis.Environmental variables that predispose to obesity in the ASC niche have a significant impact on these epigenetic modifications. Although these studies have shown the critical function that ASCs play in the process, little is known about how epigenetic modifications in ASCs under healthy or pathological situations are affected by possible modulator agents.

We were inspired to investigate the ASCs niche further by our results in the whole WAT. ASCs were separated from animal CO and HFD groups.Previous research has shown the impact of leptin on the differentiation and proliferation of ASCs [29,54,55].ASCs in WAT from HFD-obese mice that live in a persistently leptin-enriched environment are thought to work via paracrine signalling by attaching to its receptors. We discovered that both the Lepr1 and Lepr2 isoforms of the leptin receptor are expressed by ASCs.The expression of Ob-Rb, the long isoform of the leptin receptor, in ASCs from obese mice was remarkably unaffected by in vitro leptin administration, indicating stable receptor expression in spite of obesity.

The impact of leptin on acly expression in ASCs was then evaluated.We have recently shown that Acly is adversely regulated by an HFD and is significantly expressed in ASCs, which is comparable to our results in WAT [56].One research shows that during 3T3-L1 adipocyte development, ACL relates histone acetylation to cellular metabolism, and that ACL silencing affects histone acetylation and the expression of certain genes [31]. Interestingly, we found that ASCs grown with leptin showed a significant reduction in Acly expression. This result implies that the down-regulation of Acly, which was shown in our work, may be caused by long-term exposure of ASCs to a leptin-rich environment, as seen in obesityWAT.These results provide a feasible mechanism that promotes WAT dysfunction in obesity and highlight an emerging role for leptin signalling in altering gene expression patterns in ASCs. The pathophysiology of obesity-related dysfunction in WAT may be better understood by looking into the precise processes by which H3K27 acetylation and H3K27 methylation separate ASCs from adipocytes and macrophages. This information is now being expanded upon in an ongoing study. In conclusion, during a 16-week research that used a high-fat diet (HFD) to cause obesity in mice, the diet suppressed genes linked to adipokines, lipid biosynthesis, adipogenesis, and other-mogenesis while upregulating genes linked to inflammation and macrophage markers.Additionally, alterations in the expression of H3K27 enzyme modifiers as well as overall H3K27 acetylation and methylation were revealed by epigenetic analysis. These effects were somewhat offset by FOsupplementation throughout the previous eight weeks, indicating preventive benefits against obesity brought on by an HFD. In obese mice, FO also stopped decreases in ACL expression, supporting a function in maintaining histone modifications.

All things considered, FO supplementation shown potential in reducing obesity brought on by an HFD and affecting epigenetic and molecular pathways.To understand how FO influences H3K27 epigenetic markers, further study is required. These combined results show the complex effects of FO supplementation on adipose tissue's metabolic and epigenetic processes, underscoring its potential therapeutic benefit in resolving dysregulation associated with obesity.

4. MaterialsandMethods

Animals,FishOilSupplementation,andExperimentalProcedure

Eight-week-old male C57BL/6 mice were acquired from the Federal University of São Paulo's Centre for Development of Experimental Models (CEDEME) (UNIFESP). They lived in a controlled environment that was kept at 24 ±1 °C and had a 12-hour light-dark cycle.The mice were split into two groups for the first eight weeks of the 16-week experimental protocol: 12 HFD mice (26% carbs, 59% fat, and 15% proteins) and 6 control (CO) mice (9% fat, 76% carbohydrates, and 15% proteins). The HFD group was then split into two groups after the first eight weeks: HFD and HFD+FO (supplemented with fish oil), with six animals per subgroup for the remaining eight weeks. HiOmega-3 (5:1 EPA/DHA, NaturalisNutriçãoandFarmaLtd.a, São Paulo, Brazil) was the source of the fish oil supplement, which was given orally three times per week at a

dose of 2 g/kg bodyweight. The 8-week treatment period and the FO dose were chosen based on our earlier research [14,17], in which the CO and HF groups also received watervage.During the 16-week period, weekly food consumption and body weight measurements were taken. Mice were put to death via cervical dislocation and isoflurane anaesthesia after a 6-hour fast. Orbital plexus punctures were used to gather blood samples. Subcutaneous (Ing) and visceral (Epiand Rp) fat depots were removed, weighed, and processed as described below.Test of Glucose Tolerance

In the 12th week of the study, glucose tolerance tests, or GTTs, were administered.Mice were given an intraperitoneal injection of glucose (2 g kg−1BW) after a 6-hour fast. A OneTouch® glucose meter was used to test tail vein glucose at different intervals (15, 30, 45, 60, and 90 minutes). For every animal, the area under the curve was computed.WAT and SVFI Isolation

In accordance with recognised methods, the epidepot was submerged in indigestion buffer and coarsely chopped before to undergoing collagenase digestion [57]. To put it briefly, the minced samples were placed in a digestive buffer solution (which included collagenase II [Sigma Chemical, St. Louis, MO, USA] at a concentration of 1.0 mg/mL, pH7.40, and Dulbecco's modified Eagle's medium—D'MEM/HEPES20mM/BSA4%) and incubated for about 45 minutes at 37°C with orbital agitation (150 rpm). Following filtration through nylon mesh (Corning, Oneonta, NY, USA), the homogenate was centrifuged at 400× g for one minute. This produced two distinct fractions: 1. the supernatant, which contained the isolated mature adipocytes; and 2. the remaining filtrate, which contained the stromal vascular fraction (SVF), which was then centrifuged at 1500× g for ten minutes to form acellular pellet.After two washings, this particle was aspirated. The SVF was then incubated for ten minutes on ice using a red blood cell lysis buffer (Roche Diagnostics GmbH, Mannheim, Germany), followed by PBS washing and centrifugation once more.ASC isolation and leptin therapy

We carried out the process as previously mentioned [56]. In conclusion, the obtained cellular pellet (SVF) was suspended in culture medium [D'MEM Han's F-12, supplemented with 10% foetal bovine serum (FBS) and 10 mL/L penicillin/streptomycin (Gibco BRL, Oneonta, NY, USA)] and plated in 100mm culture dishes. These were then put in a 5% CO2 incubator at 37 °C (changing the medium every two days) until the confluence reached 70–80%.Following aspiration, the plates were exposed to PBS. Following their first separation (P1), the cells were reconstituted in the same culture medium, seeded onto fresh culture plates for growth, and cultivated until they once again achieved 70–80% confluence.Selecting the headherent cell population inside the SVF was the last stage in the ASC isolation process. A Neubauer chamber was used to measure the cell concentration, and the cells were then replated (P2). Cells were sown for studies (1×105 cells/well in 6-well plates (35 mm)) between passages P2 and P5, making sure that confluence did not surpass 80%.The cells were harvested from RNA and protein when the cell density reached 85–90%."teinextraction"either before to or after treatment with leptin dissolved in culture media for 24 hours at a concentration of 100 ng/mL (SigmaChemical, St. Louis, MO, USA).One sample was tallied for each pooled cell.Using the Trizol reagent (InvitrogenLifeTechnologies, Walthamm, MA, USA), total RNA was extracted from the whole Epi adipose depot or ASCs using RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR).RNA quality was evaluated using NANODROP One microvolume UVVis Spectrophotometers (Thermo Scientific, Walthamm, MA, USA) to measure ratios at 260/280 and 260/230 nm.The Superscript III cDNA kit (Thermo Scientific, USA) was used to accomplish reverse transcription to cDNA. According to a previously published research [56,58], the Rotor gene (Qiagen, Dusseldorf, Germany) and SYBR Green fluorescent dye were used in a quantitative real-time polymerase chain reaction (PCR) to measure gene expression. The 2ΔΔCt technique was used to analyse real-time PCR data, and the findings were reported as the ratio of target gene expression to housekeeping genes (Gapdhand36b4).In Table 2, certain primer sequences are listed.

Table2. Senseandantisenseprimersequencesusedforq RT-PCR.

Gene	5′Primer(5′-3′)-Sense	3′Primer(5′-3′)-Antisense
Gapdh	AAATGGTGAAGGTCGGTGTG	TGAAGGGGTCGTTGATGG
Ep300(p300)	GTTGCTATGGGAAACAGTTATGC	TGTAGTTTGAGGTTGGGAAGG
Ezh2	CAGGATGAAGCAGACAGAAGAGG	TCGGGTTGCATCCACCACAAA
Kdm6a	GCTGGAACAGCTGGAAAGTC	GAGTCAACTGTTGGCCCATT
Kdm6b	CCTATTATGCTCCTGGGACA	TACGGCTTCCTCACTGTCGT
Crebbp(Cbp)	GACCGCTTTGTTTATACCTGC	TCTTATGGGTGTGGCTCTTTG
Acly	TCCGTCAAACAGCACTTCC	ATTTGGCTTCTTGGAGGTG
36b4(Rplp0)	TAAAGACTGGAGACAAGGTG	GTGTACTCAGTCTCCACAGA
Lepr1	CAGAATGACGCAGGGCTGTA	GCTCAAATGTTTCAGGCTTTTGG
Lepr2	ATTAATGGTTTCACCAAAGATGCT	AAGATCTGTAAGTACTGTGGCAT

Gapdh,Glyceraldehyde-3-phosphateDehydrogenase;*Ep300(p300)*,E1Abindingproteinp300;*Ezh2*, Enhancerofzest2polycombrepressivecomplex2subunit;*Kdm6a*,Lysine(K)-specificdemethylase 6A;*Kdm6b*,Lysine(K)-specificdemethylase6B;*Crebbp*,CREBbindingprotein;*Acly*,ATPcitrately- ase; *36b4 (Rplp0)*, ribossomal protein lateral stalk subunit P0; *Lepr1*, leptin receptor 1; *Lepr2*, Leptin receptor 2.

PCRArrayGeneExpressionAnalysis

ForPCRarraygeneexpressionanalysis,RNAwasisolatedusinganRNAextraction kitfollowingthemanufacturer'sinstructions,andtheassaywasconductedaspreviously described [59]. Briefly, cDNA and RT2 SYBR®Green qPCR Mastermix (Cat. No. 330529) were utilized on a Custom Mouse RT2 Profiler PCRArray (CLAM30774R; Qiagen) con- sistingof84genes.Thisarrayallowedustoassesstheexpressionpatternofgenesencod- ingpro/anti-adipogenic,pro/anti-lipogenicandlipolytic,pro/anti-browning,adipokines, receptors, and components of adipocyte transduction pathways (see Table 3). CT values were exported and uploaded to the manufacturer's data analysis web portal at http://www.qiagen.com/geneglobe. Samples were categorized into control and test groups, and CT values were normalized based on a manual selection of reference genes. Fold Change wascalculated usingthe $2^{\Delta\Delta Ct}$method via the data analysisweb portal(and exported at GeneGlobe®, Qiagen).

Table3. ListofselectedgenesinCustomMouseRT2ProfilerPCRArray.

Pathways	Genes
Adipokines	*Adipoq(Acrp30),Cfd(Adipisin),Lep(leptin),Retn(Resistin)*
Lipasesandlipogenic enzymes	*Acaca(Acc1),Gpd1(glycerol-3-phosphatedehydrogenase1(soluble),Lipe(HSL),Scd1 (stearoyl CoA desaturase), Lpl, Pnpla2 (Atgl), Lipin 1, Pck1 (phosphoenolpyruvate carboxykinase 1), Fasn*
Pro-adipogenesis	*Cebpa,Cebpb,Cebpd,Pparg(PPARgamma2),Srebf1,Fabp4(aP2),Pilin1,Fgf2(bFGF), Fgf10, Jun (c-jun ou AP1), Lmna (Lamini A), Sfrp1 (secreted frizzled-related protein1), Slc2a4 (Glut4), Klf15, Klf4*
Anti-adipogenesis	*Adrb2,Cdkn1a(p21Cip1,Waf1),Cdkn1b(p27Kip1),Ddit3(Gadd153,Chop),Dlk1(Pref1), Foxo1, Ncor2, Shh, Sirt1, Wnt1, Wnt3a, Gata2, Klf*
Pro-Browning,fattyacid thermogenesis, and oxidation	*Bmp7,Cidea,Cpt1b,Creb1,Dio2,Elovl3,Foxc2,Mapk14(p38alpha),Nrf1,Ppara,Ppard, Ppargc1a(Pgc1alpha), Ppargc1b(Perc, Pgc1beta), Prdm16,Sirt3, Src, Tbx1, Tfam, Ucp1, Wnt5a*
Anti-Browning	*Ncoa2,Nr1h3,Rb1,Wnt10b*
Adipokinesreceptors	*Lepr,Adipor2,Adrb1*
Cytokines,growthfactors, Andsignaltransduction	*Ccl2(MCP1),Cxcl10,Ifng,Il1b,Il4,Il6,Il10,Il12b,Il13,Tgfb1,Tnf,Insr,Irs1,Irs2,Akt2, Ptpn1(PTP1B),Ikbkb(IKKbeta),Mapk8(JNK1),Nfkb1,Pik3r1(p85alpha),Irf4,Retnla (Resistin-like alpha, Fizz1), Cd68*

Acetyl-Coenzyme A carboxylase alpha, or Acaca (Acc1); Actin, beta—Actinb or β-actin; Akt2—Thymomaviral proto-oncogene2; Adipoq—Adiponectin; Adipor2—Adiponectin receptor2; Adrb1—Adrenergic receptor, beta1; Adrb2—Adrenergic receptor, beta2;Beta-2 microglobulin, or B2m, and bone morphogenetic protein 7, or Bmp7, Cd68—CD68 antigen; Ccl2—Chemokine (C-C motif) ligand 2; Cyclin-dependent kinase inhibitor 1A (P21) is known as Cdkn1a; Cyclin-dependent kinase inhibitor 1B is known as Cdnk1b. Cebpa or C/EBPα—CCAAT/enhancerbindingprotein (C/EBP), alpha; Cebpbor C/EBPβ—CCAAT/enhancer bindingprotein (C/EBP), beta; Cebpd—CCAAT/enhancerbindingprotein (C/EBP), delta;Cidea—Cell death-inducing DNA fragmentation factor, alpha subunit-like effector A; Cfd—Complement factor D (adipsin); Cpt1b; CAMP response element-binding protein 1; Creb1; Cxcl10; DNA-damage-inducible transcript 3; Dio2—Deiodinase, iodothyronine, type II; Dlk1 (Pref-1)—Delta-like 1 homolog (Drosophila); Elongation of extremely long chain fatty acids (Yeast)-like 3 (FEN1/Elo2, SUR4/Elo3);Fasn—Fatty Acid Synthase; Fabp4(aP2)—Fatty Acid Binding Protein4, adipocyte;Fibroblast growth factor 10 (Fgf10) and Fibroblast growth factor 2 (Fgf2)Gapdh—glyceraldehyde-3-phosphate dehydrogenase; Gata2—GATAbindingprotein2; Gpd1—glycerol-3-phosphate dehydrogenase1 (soluble); Ifng—interferongamma; Foxc2—Forkhead box C2; Foxo1—Forkhead box O1;Ikbkb (IKKbeta)—KappaB kinase beta inhibitor; Interleukin 10 (Il10), Interleukin 12b (Il12b), Interleukin 13 (Il13), Interleukin 1beta (Il1b), Interleukin four (Il4), and Interleukin six (Il6)Irs1—Insulin receptor substrate 1; Irs2—Insulin receptor substrate 2; Jun—Jun on-cogene; Insr—Insulin receptor; Irf4—Interferon regulatory factor 4;Keller-like factor 15 (Klf15), Keller-like factor 2 (lung), and Keller-like factor 4 (gut)Lmna—Lamin A; Lipe (HSL)—Lipase, hormone-sensitive; Lep—leptin; Lepr—Leptin receptor; Lpl—lipoprotein lipase; Lpin1—lipin 1; Mitogen-activated protein kinase 14 (Mapk14); Mitogen-activated protein kinase 8 (Mapk8 (Jnk1)); nuclear receptor co-repressor 2 (Ncoa2); and nuclear receptor co-repressor 2 (Ncor2); Nr1h3—Nuclear receptor subfamily1, group H, member 3; Nrf1—Nuclear respiratory factor 1; Pck1—Phosphoenolpyruvate carboxykinase 1 cytosolic; Nfkb1—Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1,p105; Plin1—Perilipin 1; Ppara—Peroxisome proliferator activated receptororalpha; Ppard—Peroxisome proliferator activator receptordelta; Pik3r1—Phosphatidylinositol 3-kinase, regulatory subunit, polypeptide 1 (p85 alpha);Ppargc1a—Peroxisome proliferative activated receptor, gamma, coactivator 1 alpha; Ppargc1b—Peroxisome proliferative activated receptor, gamma, coactivator 1 beta; PpargorPPARγ—Peroxisome proliferator activated receptor, gamma; Rb1—Retinoblastoma1; RTC—Reverse Transcription Control; Prdm16—PR domain containing 16; Ptpn1—Protein tyrosine phosphatase, non-receptortype1; Retn—Resistin; Retnla—Resistin-likealpha; Scr stands for rous sarcoma oncogene; Scd1 for Stearoyl-Coenzyme A desaturase 1; Secreted Frizzled-Related Protein 1 (Sfrp1) and Sonichedgehog (Shh)(S. cerevisiae) Sirt1—Sirtuin1 (silentmating type information regulation 2, homolog)1; Sirt3—Sirtuin3 (silentmating type information regulation 2, homolog) 3; Slc2a4—Glut4—Solute carrier family 2—member 4 of the facilitated glucose transporter; Tbx1—T-box 1; Tfam—Mitochondrial transcription factor A; Srebf1—Sterol regulatory element binding transcription factor 1; Tnf—tumor necrosis factor; TgA1—transforming growth factor, beta 1; Ucp1: Uncoupling protein 1 (proton carrier, mitochondria); Wingless-related MMTV integration site 1 is designated as Wnt1; Wingless-related MMTV integration site 10b is designated as Wnt10b; Wingless-related MMTV integration site 3A is designated as Wnt3a; and Wingless-related MMTV integration site 5A is designated as Wnt5a.WesternBlot

Proteins from ASCs or EpiWAT depots (wholet tissues) were converted to nitrocellulose membranes (0.2µm) after being resolved by 12% or 15% sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (PAGE).After being blocked with 5% albumin for two hours at room temperature, the membranes were treated for a whole night at 4 °C with primary antibodies (anti-H3K27ac #ab4729, H3K27me3 #sab5700166, anti-ACL #ab40793, anti-LeptinReceptor #ab5593,Abcam, Waldhamm, MA, USA). After washing, the membranes were incubated for one hour at room temperature with secondary horse serum peroxidase-conjugated anti-rabbit IgG (CellSignaling®, Danvers, MA, USA—#7074) antibodies. An enhanced chemiluminescence Western Blotting detection kit (ECL Prime Western Blotting System, Amersham Biosciences®, Amersham, UK) was used to visualise protein blots. As an endogenous standard, beta-actin levels (Cell Signaling®, Danvers, MA, USA—#4967L) were used.Utilising Scion Image software (Scion Corporation, Frederick, MD, USA), protein quantification was examined.Ponceau's expression of constitutive beta-actin and total protein rectified all of the findings, which were expressed in relation to control group levels.Analytical Statistics As shown in the figures, the data were analysed using one-way analysis of variance (ANOVA), which was followed by either the Student's t-test or Tukey's post-test for group comparisons.The findings are displayed as mean±standard error of the mean (SEM).Significant differences were defined as $p < 0.05$. Version 9.1.2 of GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA) was used to conduct the statistical analysis. With tabulation and analysis in databases including Gene Ontology Analysis and UniProt, the PCRArray data were examined using the RT2ProfilerPCRArrayDataAnalysis Software version 3.5 (SABiosciences, Frederick, MD, USA).

5. Conclusions

Our research emphasises how important epigenetic changes—more especially, H3K27ac and H3K27me3—are in WAT dysfunction-related obesity. These changes are likely triggered by the inflammatory environment, which includes obesity-rich leptin in obesity. They may also impact gene expression patterns and exacerbate the chronic inflammatory state in a positive feedback loop by influencing adipogenesis and/or adipocyte maturation. Considering the paucity of research on the relationship between WAT epigenetics and FO therapy, findings Findings suggest a good strategy for managing issues connected to obesity by highlighting the possible involvement of FO in modifying these epigenetic variables.In order to shed more light on how this dysregulation contributes to the persistent inflammatory states observed in obesity, as well as disease pathogenesis and possible therapeutic targets, future research will concentrate on examining the reciprocal relationship between inflammation and H3K27 epigenetic marks during adipogenesis.

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