FAT ACCUMULATING FISH CELLS AND A METHOD FOR PREPARING SAME

CROSS REFERENCE TO RELATED APPLICATIONS

[001] This application claims the benefit of priority of U.S. Provisional Patent Application No. 63/550,640, titled "FAT ACCUMULATING FISH CELLS AND A METHOD FOR PREPARING SAME", filed on 7 February 2024, the contents of which are incorporated herein by reference in their entirety.

FIELD OF INVENTION

[002] The present invention is directed to, *inter alia*, compositions of cultured meat and seafood and methods of producing the same.

BACKGROUND

[003] Cultured meat, also known as lab-grown, engineered, or clean meat, is produced from cell culture using tissue engineering techniques and is a prominent alternative for traditional meat production using live animals. In the last decade, this notion gained increased attention in public opinion, popular media, investors, and the scientific community, notably after the production of the first lab-grown cultured beef burger. Food produced using animals is considered to be inefficient, as the animals consume large amounts of food throughout their lives, of which 80-90% of the calories are wasted on the animal's metabolism and the production of non-edible tissues.

[004] In addition, the world's demand for meat is expected to double by 2050, meaning traditional meat production systems are not sustainable. Compared to several meat sources, cultured meat is estimated to decrease 7-45% of energy use, 78-96% of the greenhouse gas (GHG) emissions, 99% of land use and 82-96% of water use.

[005] Similarly to the livestock industry, fish consumption is vulnerable to a number of problems including overfishing and by-catch as well as pollution caused by fisheries.

[006] There is still a great need for a method for producing fish-based meat products for consumption that is more efficient, safer, and healthier than the current methods of production.

SUMMARY

[007] According to the first aspect, there is provided a cultured fish cell comprising an increased amount of polyunsaturated fatty acids (PUFA) and reduced amount of monounsaturated fatty acids (MUFA) compared to a control fish cell.

[008] According to another aspect, there is provided a method for inducing fat accumulation in a fish cell, the method comprising contacting a fish cell with an effective amount of a composition comprising a fat accumulation inducing agent; and (ii) a carrier, wherein the fat accumulation inducing agent consists essentially of a free fatty acid and a peroxisome proliferator-activated receptor gamma (PPARγ) agonist, thereby inducing fat accumulation in the fish cell.

[009] According to another aspect, there is provided a composition comprising the cultured fish cell of the invention, being an edible composition.

[010] According to another aspect, there is provided a food product, comprising the composition of the invention.

[011] In some embodiments, the control fish cell is derived from a wild caught or farm raised fish of the same species as the cultured fish cell.

[012] In some embodiments, the polyunsaturated fatty acids comprise omega-3 PUFA, omega-6 PUFA, omega-9 PUFA, or any combination thereof.

[013] In some embodiments, the cultured fish cell comprises omega-3 PUFA in an amount of 20% to 80% by weight of fatty acids in the cultured fish cell.

[014] In some embodiments, the cultured fish cell is characterized by omega-3 PUFA to omega-6 PUFA weight per weight ratio (w/w) ranging between 8:1 (w/w) and 15:1 (w/w).

[015] In some embodiments, the cell is a fibroblast, a myoblast, or a combination thereof.

[016] In some embodiments, the fish belongs to the subfamily *Scombrinae*.

[017] In some embodiments, the fish belons to the genus *Thunnus*.

[018] In some embodiments, the fish is of a species selected from the group consisting of: *T. maccoyii*, *T. orinetalis*, *T. thynnus*, *T. tonggol*, *T. alalunga*, and *T. albacares*.

[019] In some embodiments, the free fatty acid is an unsaturated fatty acid.

[020] In some embodiments, the free fatty acid comprises a fatty acid chain of up to 18 carbon atoms.

[021] In some embodiments, the free fatty acid is selected from the group consisting of: α-Linolenic acid, oleic acid, linoleic acid, and any combination thereof.

[022] In some embodiments, the composition comprises the free fatty acid in a concentration ranging between 50 μM and 350 μM.

[023] In some embodiments, the PPARγ agonist is selected from the group consisting of: Indomethacin, Rosiglitazone, L-α-Phosphatidylcholine, and any combination thereof.

[024] In some embodiments, the composition comprises any one of: (a) the Indomethacin in a concentration ranging between 1 nM and 10 nM; (b) the Rosiglitazone in a concentration ranging between 5 μM and 20 μM; (c) the L-α-Phosphatidylcholine in a concentration ranging between 1 ng/ml and 20 ng/ml; and (d) any combination of (a)-(c).

[025] In some embodiments, the fat accumulation inducing agent consists essentially of α-Linolenic acid in a concentration ranging between 100 μM and 300 μM in the composition, and Rosiglitazone in a concentration ranging between 7 μM and 15 μM in the composition.

[026] In some embodiments, the fat accumulation inducing agent consists essentially of oleic acid in a concentration ranging between 100 μM and 300 μM in the composition, and Rosiglitazone in a concentration ranging between 7 μM and 15 μM in the composition.

[027] In some embodiments, the composition is devoid of: 3-isobutyl-1-methylxanthine (IBMX), insulin, dexamethasone, or any combination thereof.

[028] In some embodiments, the method further comprises contacting the fish cell with an effective amount of fibroblast growth factor (FGF).

[029] In some embodiments, the FGF is salmon FGF.

[030] In some embodiments, the method further comprises culturing the fish cell after the contacting.

[031] In some embodiments, the contacting, culturing, or both, is in a temperature ranging between 18 °C and 26 °C.

[032] In some embodiments, the method is an ex vivo or an in vitro method.

[033] Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or

materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

[034] Further embodiments and the full scope of applicability of the present invention will become apparent from the detailed description given hereinafter. However, it should be understood that the detailed description and specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE FIGURES

[035] **Figs. 1A-1B** include fluorescent micrographs and brightfield micrographs showing that Isobutyl methylxanthine (IBMX), insulin, dexamethasone, and lipid mixture drive fat accumulation (FA) in Mackerel myoblasts, Yellowtail myoblasts, and bluefin tuna (BFT) fibroblast cells, but not in BFT myoblasts. (**1A**) Representative fluorescent images of Mackerel myoblasts, Yellowtail myoblasts, BFT fibroblast cells, and BFT myoblasts cells following three days in regular proliferation culture conditions (Control, Left panel) and following three days in FA induction medium containing IDIL (IBMX, dexamethasone, insulin, and lipid mixture; as detailed in Saad et al., 2023; IDIL, Right panel). Neutral fats accumulated in lipid droplets are stained with either BODIPY (in green) or Nile Red (in red, in Yellowtail cells), and nuclei are stained for contract with DAPI (in blue) or Nuclear green (in green, in Yellowtail cells) for visualization. Fats accumulated in the cells are visible as punctate in IDIL-treated Mackerel myoblasts, BFT fibroblasts, and Yellowtail myoblasts. However, IDIL did not lead to FA in BFT myoblasts. (**1B**) Representative images of BFT myoblast cells following three days in regular proliferation culture conditions (Control) and following seven days with different treatments – IDIL, human insulin at 10 μg/ml, and lipid mixture at 1:100 dilution are sufficient to cause a toxic effect in BFT myoblast cells. Human insulin at 1μg/ml and lipid mixture at 1:1,000 dilution does not produce a toxic effect. Still, treatment with IDIL-reduced (1 μg/ml for human insulin and 1:1,000 for the lipid mixture) did not lead to FA in BFT myoblasts.

[036] **Figs. 2A-2E** include fluorescent micrographs, micrographs, and vertical bar graphs showing that peroxisome proliferator-activated receptor (PPAR) gamma (PPARγ) agonists alone nor with chemically defined lipid concentrate (CDLC) fail to drive FA in BFT cells. (**2A**) Representative images of BFT fibroblast cells and BFT myoblasts cells following three days in

regular proliferation culture conditions (Control) and following three days in FA induction medium containing CDLC as a lipid scours with Rosiglitazone, Indomethacin, or L-α-Phosphatidylcholine (L-α-PSC). FA was visualized by cell morphology or by staining neutral fats with BODIPY (in green) and DAPI (blue, nuclear staining). No FA was observed in BFT fibroblasts (Top panel) or BFT myoblasts (bottom panel) following FA induction using CDLC. (**2B**) The toxicity of different concentrations of three agonists to BFT myoblast cells was evaluated. Cells were treated with increasing concentrations (5 and 10 μM) of Rosiglitazone for 11 days. Representative images of treated BFT myoblasts show no toxic effect. (**2C**) Further toxicity was measured for Indomethacin 5, 10, and 25 nM) and L-α-Phosphatidylcholine (1, 5, 10, and 20 ng/ml) using the PrestoBlue viability reagent. Viability was recorded on day 4, day 7, and day 11. Results are normalized to control cells for relative viability. Results indicate slight viability loss with Indomethacin 10-25 nM from day seven, and no toxic effect with L-α-PSC. (**2D**). fluorescence-activated cell sorting (FACS) analysis for FA using BODIPY staining shows that neither of the PPARΓ agonists induced FA in BFT fibroblast cells. The Bars represent the percentage of cells positive for BODIPY within the treated cell population. Treatments include Control, 10 μM Rosiglitazone ('Ros'), 5 nM Indomethacin ('Indomethacin'), and 12 ng/ml L-α-Phosphatidylcholine (L-α-PSC). (**2E**) Similarly, BODIPY analysis shows minor levels of FA in BFT myoblast cells following treatment with 10 μM Rosiglitazone ('Ros'), 5 nM Indomethacin ('Indomethacin'), or 12 ng/ml L-α-Phosphatidylcholine (L-α-PSC).

[037] **Figs. 3A-3D** include fluorescent micrographs, and graphs showing that PPARΓ agonists and oleic or alpha-linolenic acid drive FA in BFT myoblast cells. (**3A**) Representative fluorescent images of BFT myoblasts following three days of FA treatment with 200 μM oleic acid (OA) + 10 μM Rosiglitazone (Ros), 200 μM OA + 5 nM Indomethacin, and 200 μM OA + 12 ng/ml L-α-Phosphatidylcholine (L-α-PSC). Lipid droplets are stained with BODIPY for visualization. Results indicate FA induction with all three PPARΓ agonists, while the combination of Rosiglitazone with OA presents the most pronounced effect. (**3B**) BODIPY analysis shows FA following different combination treatments of BFT myoblasts with: 200 μM OA with 10 μM Rosiglitazone, 300 μM OA with either one of the three PPARΓ agonists ('Ros', Indomethacin, and L-α-PSC), 200 μM alpha linolenic acid (aLA), and 300 μM aLA with 10 μM Rosiglitazone. The bars represent the percentage of positive cells for BODIPY. The results show that 300 μM of fatty acids lead to better FA outcomes, Rosiglitazone performs better than other agonists regarding FA, and 300 μM aLA + 10 μM Rosiglitazone leads to optimal FA performance. (**3C**) Triglyceride quantification (μg/ml) of BFT myoblasts after 24 h, 48 h, and

72 h of FA treatment with 10 μM Rosiglitazone and 300 μM OA. (**3D**) Triglyceride quantification of BFT myoblast cells following three days of FA treatment with 10 μM Rosiglitazone and 300 μM aLA as a lipid source.

[038] **Figs. 4A-4C** include fluorescent micrographs and graphs showing that agonists and oleic or alpha-linolenic acid drive FA in BFT fibroblast cells. (**4A**) Representative fluorescent images of BFT fibroblasts following three days of FA treatment with 300 μM OA + 10 μM Rosiglitazone, 300 μM OA + 5 nM Indomethacin, 300 μM OA + 12 ng/ml L-α-Phosphatidylcholine, and 300 μM aLA + 10 μM Rosiglitazone. Lipid droplets are stained with BODIPY (in green) for visualization. Results indicate FA induction with all three PPARΓ agonists, while the combination of Rosiglitazone and Indomethacin with OA presents the most pronounced effect. (**4B**) BODIPY analysis by FACS shows FA following different combination treatments of BFT fibroblasts with either 200 μM OA with 10 μM Rosiglitazone, 300 μM OA with either of the three PPARΓ agonists, and 300 μM aLA with 10 μM Rosiglitazone. The bars present the percentage of positive cells for BODIPY. Results show that 300 μM OA leads to better FA outcomes, Rosiglitazone performs better than other agonists regarding FA, and 300 μM aLA + 10 μM Rosiglitazone leads to optimal FA performance. (**4C**) Triglyceride quantification (μg/ml) of BFT fibroblasts after 24 h, 48 h, and 72 h of FA treatment with control, 10 μM Rosiglitazone and 300 μM OA, and 10 μM Rosiglitazone and 300 μM a-LA (72 hours Only).

[039] **Figs. 5A-5E** include graphs and fluorescent micrographs showing that either oleic acid or alpha-linolenic acid, drives FA in BFT myoblast and fibroblast cells, even in the absence of PPARΓ agonists. (**5A**) BODIPY analysis by FACS shows FA following OA or aLA treatment in BFT myoblasts. Cells were treated with only fatty acid sources: 200 or 300 μM OA, 200 or 300 μM aLA for BFT myoblasts. The results show FA with the two fatty acids sources, with aLA delivering higher levels of FA, and indicate that FA in BFT myoblast cells can occur in the presence of only a fatty acid source. (**5B**) Triglyceride quantification (μg/ml) of BFT myoblasts after 24h, 48h, and 72h of FA treatment with control, 300 μM OA, and 300 μM aLA (72 hours Only). (**5C**) BODIPY analysis by FACS shows FA following OA or aLA treatment in BFT fibroblast cells. Cells were treated with only fatty acid: either 300 μM OA or 300 μM aLA. The results show FA with the two fatty acids sources, with aLA delivering higher levels of FA. (**5D**) Representative fluorescent images of BFT fibroblasts following three days of FA treatment with 300 μM OA or 300 μM aLA. Lipid droplets are stained with BODIPY (in green) for visualization. The results indicate FA induction with both OA and aLA, with aLA delivering

higher levels of FA. This indicates that FA in BFT fibroblast cells can occur with only a fatty acid source and without a PPARΓ agonist. (**5E**) Triglyceride quantification (µg/ml) of BFT fibroblasts after 24 h, 48 h, and 72 h of FA treatment with control, 300 µM OA, and 300 µM aLA.

[040] **Figs. 6A-6B** includes fluorescent micrographs showing that the combination of rosiglitazone with any one of alpha-linolenic acid and oleic acid, drives FA in *Tuna albacore* (*Thunnus alalunga*) myoblast and Yellowtail (*Seriola dumerili*) fibroblast cells. Representative BODIPY images of Albacore myoblast (**6A**) and Yellowtail fibroblast (**6B**) cells following three days in regular proliferation culture conditions (Control) and following 24 hours in FA induction medium containing treatments of Rosiglitazone (10 µM) with either OA (200 µM) aLA (200 µM) Neutral fats accumulated in lipid droplets are stained with BODIPY. Fats accumulated in the cells are visible as punctate dots. In both (**6A-6B**), top panel represents 10× image magnifications, and bottom panel represents images with 20× magnifications.

[041] **Figs. 7A-7B** include graphs showing Fatty acid methyl esters (FAME) analysis reflected as mg fatty acid (FA)/100 mg sample (**7A**) and % FA class of total FAs (**7B**) in fibroblasts induced for fat accumulation according to the method of the invention. Further presented are controls derived from fresh tuna cuts including Toro, and Akami.

DETAILED DESCRIPTION

[042] According to the first aspect, there is provided a cultured fish cell having an increased amount of polyunsaturated fatty acids (PUFA) and reduced amount of monounsaturated fatty acids (MUFA) compared to a control fish cell.

[043] In some embodiments, the control fish cell comprises or is derived from a wild caught or farm raised fish of the same species as the cultured fish cell. In some embodiments, a control comprises a fish cell not induced according to the method of the invention. In some embodiments, a control comprises a fish cell derived from a fresh cut of a wild caught or farm raised fish of the same species as the cultured fish cell. In some embodiments, the fresh cut comprises or is Toro, Akami, or both. In some embodiments, a control comprises at least one cell being derived from Toro, Akami, or both.

[044] As used herein, the expression "fresh cut (such as of a fish), covers any portion that can be cut from a fish and is known to a skilled artisan. In some embodiments, a fresh cut of a fish (such as a tuna fish) is selected from: Toro, Akami, Noten, Hoho-Niku (Cheek), Kama-toro

(Back cheek), Harakami-otoro, Haranaka-chutoro, Harashimo, Sekami, Senaka, Seshimo, or any combination thereof.

[045] In some embodiments, polyunsaturated fatty acids comprise omega-3 PUFA, omega-6 PUFA, omega-9 PUFA, or any combination thereof. In some embodiments, polyunsaturated fatty acids comprise omega-3 PUFA and omega-6 PUFA. In some embodiments, polyunsaturated fatty acids comprise omega-3 PUFA and omega-9 PUFA. In some embodiments, polyunsaturated fatty acids comprise omega-6 PUFA and omega-9 PUFA. In some embodiments, polyunsaturated fatty acids comprise omega-3 PUFA, omega-6 PUFA, and omega-9 PUFA.

[046] In some embodiments, the cultured cell is a fibroblast, a myoblast, or a combination thereof. In some embodiments, the fish belongs to the subfamily *Scombrinae*. In some embodiments, the fish belons to the genus *Thunnus*. In some embodiments, the fish is of a species selected from the group consisting of: *T. maccoyii*, *T. orinetalis*, *T. thynnus*, *T. tonggol*, *T. alalunga*, and *T. albacares*.

[047] In some embodiments, increased is 5-100%, 10-100%, 25-100%, 50-100%, 75-100%, 50-200%, 50-500%, 75-600%, 250-500%, or 150-1,000% increase compared to the control. Each possibility represents a separate embodiment of the invention.

[048] In some embodiments, reduced is 1-100%, 10-100%, 20-100%, 50-100%, 75-100%, or 90-100% reduction compared to the control. Each possibility represents a separate embodiment of the invention.

[049] In some embodiments, the cultured fish cell comprises a PUFA (including any one of Omega-3 PUFA, Omega-6 PUFA, Omega-9 PUFA, or any combination thereof) in an amount of at least 20%, 30%, 40%, 50%, 60%, 70%, or 80% of the total fatty acids content, or any value and range therebetween. In some embodiments, the cultured fish cell comprises a PUFA (including any one of Omega-3 PUFA, Omega-6 PUFA, Omega-9 PUFA, or any combination thereof) in an amount of 20-80%, 30-80%, 40-80%, 50-80%, 60-80%, or 70-90% of the total fatty acids content. Each possibility represents a separate embodiment of the invention.

[050] In some embodiments, the cultured fish cell comprises an Omega-3 PUFA, in an amount of at least 20%, 30%, 40%, 50%, 60%, 70%, or 80% of the total fatty acids content, or any value and range therebetween. In some embodiments, the cultured fish cell comprises an Omega-3 PUFA in an amount of 20-80%, 30-80%, 40-80%, 50-80%, 60-80%, or 70-90% of the total fatty acids content. Each possibility represents a separate embodiment of the invention.

[051] In some embodiments, the cultured fish cell comprises an Omega-6 PUFA, Omega-9 PUFA, or both in an amount of at least 3%, 4%, 5%, 6%, 7%, or 8% of the total fatty acids content, or any value and range therebetween. In some embodiments, the cultured fish cell comprises an Omega-6 PUFA, Omega-9 PUFA, or both, in an amount of 3%, 4%, 5%, 6%, 7%, or 8% of the total fatty acids content. Each possibility represents a separate embodiment of the invention.

[052] In some embodiments, the cultured fish cell comprises a MUFA in an amount of not more than 8%, 9%, 10%, 20%, 25%, or 30% of the total fatty acids content, or any value and range therebetween. In some embodiments, the cultured fish cell comprises MUFA, in an amount of 1-30%, 1-25%, 1-20%, 1-15%, or 1-10% of the total fatty acids content. Each possibility represents a separate embodiment of the invention.

[053] In some embodiments, the cultured fish cell comprises a saturated fatty acid or a plurality thereof in an amount of 1-15%, 1-20%, 1-23%, 10-20%, or 15-20% of the total fatty acids content. Each possibility represents a separate embodiment of the invention.

[054] In some embodiments, the cultured fish cell comprises omega-3 PUFA in an amount of at least 20%, at least 30%, at least 40%, at least 50%, or at least 60% by weight of fatty acids in the cultured fish cell, or any value and range therebetween. In some embodiments, the cultured fish cell comprises omega-3 PUFA in an amount of 20% to 80%, 30% to 80%, 40% to 80%, 50% to 80%, 60% to 80%, 20% to 70%, 30% to 70%, 40% to 70%, or 50% to 70% by weight of fatty acids in the cultured fish cell. Each possibility represents a separate embodiment of the invention. In some embodiments, the amount of omega-3 PUFA is by weight of the total amount or weight of fatty acids in the cultured fish cell.

[055] In some embodiments, the cultured fish cell is characterized by omega-3 PUFA to omega-6 PUFA weight per weight ratio (w/w) of at least 7:1 (w/w), at least 8:1 (w/w), at least 9:1 (w/w), at least 10:1 (w/w), at least 12:1 (w/w), at least 15:1 (w/w) or any value and range therebetween. In some embodiments, the cultured fish cell is characterized by omega-3 PUFA to omega-6 PUFA w/w ranging between 7:1 (w/w) and 15:1 (w/w), 8:1 (w/w) and 15:1 (w/w), 9:1 (w/w) and 15:1 (w/w), 7:1 (w/w) and 12:1 (w/w), 8:1 (w/w) and 12:1 (w/w), 9:1 (w/w) and 11:1 (w/w), 8:1 (w/w) and 10:1 (w/w), or 7:1 (w/w) and 11:1 (w/w). Each possibility represents a separate embodiment of the invention.

[056] As used herein, the term “saturated fatty acid” refers to a type of fatty acid that has no double bonds between the carbon atoms of the fatty acid chain. This means that the carbon

chain is fully "saturated" with hydrogen atoms. Saturated fatty acids are typically solid at room temperature and are found in animal fats and some plant oils.

[057] As used herein, the term "unsaturated fatty acid" refers to a type of fatty acid that contains one or more double bonds between the carbon atoms in the fatty acid chain. These double bonds can cause the chain to bend, affecting the physical properties of the fatty acid. Unsaturated fatty acids are typically liquid at room temperature and are commonly found in plant oils and fish. They are further categorized into monounsaturated fatty acids (with one double bond) and polyunsaturated fatty acids (with two or more double bonds).

[058] As used herein, the terms "polyunsaturated fatty acid" or "PUFA" refers to a type of unsaturated fatty acid that contains two or more double bonds in its carbon chain. These double bonds can cause the chain to bend, affecting the physical properties of the fatty acid. Polyunsaturated fatty acids are typically liquid at room temperature and are found in high concentrations in fish, nuts, seeds, and plant oils. They are essential fats, meaning the body cannot produce them, and they must be obtained through the diet. PUFAs include omega-3 and omega-6 fatty acids, which are important for various bodily functions and maintaining health.

[059] As used herein, the terms "monounsaturated fatty acid" or "MUFA" refers to a type of unsaturated fatty acid that contains one double bond in its carbon chain. This single double bond can cause the chain to bend, affecting the physical properties of the fatty acid. Monounsaturated fatty acids are typically liquid at room temperature and are commonly found in plant oils, such as olive oil, as well as in nuts and avocados. They are considered beneficial for health, as they can help reduce bad cholesterol levels and provide nutrients to help develop and maintain the body's cells.

[060] As used herein, the term "plurality" refers to any integer being equal to or greater than 2.

[061] According to another aspect, there is provided a method for inducing fat accumulation in a cell of a fish.

[062] According to another aspect, there is provided a method for inducing fat accumulation in a cell of a bluefin tuna. According to another aspect, there is provided a method for producing a fat accumulating bluefin tuna cell.

[063] In some embodiments, inducing is with reduced toxicity. In some embodiments, toxicity is cellular toxicity. In some embodiments, the method comprises inducing fat accumulation with reduced toxicity or cellular toxicity to the cell induced to accumulate fat according to the claimed invention.

[064] In some embodiments, the method comprises inducing fat accumulation in a cell in conjunction with maintaining and/or preserving fitness, viability, survival, or any combination thereof, of the induced cell. In some embodiments, maintaining or preserving refers to the induced cell having fitness, viability, survival, or any combination thereof, being essentially similar to a control cell (such as not being induced according to the method of the invention). In some embodiments, essentially similar refers to being at least 80%, 90%, 95%, or 99% of the control, or any value and range therebetween. In some embodiments, being essentially similar to control is being identical to the control (e.g., 100% similar). Each possibility represents a separate embodiment of the invention.

[065] In some embodiments, the method comprises contacting a cell obtained or derived from a fish with an effective amount of a composition comprising: (i) a fat accumulation inducing agent; and (ii) a carrier, thereby inducing fat accumulation in the cell of a fish.

[066] In some embodiments, the method comprises contacting a cell obtained or derived from a bluefin tuna with an effective amount of a composition comprising: (i) a fat accumulation inducing agent; and (ii) a carrier, thereby inducing fat accumulation in the cell of a bluefin tuna.

[067] In some embodiments, the composition is a fat accumulation composition. In some embodiments, the composition is a fat accumulation inducing composition. In some embodiments, the composition is a manipulation composition.

[068] In some embodiments, the method comprises providing or obtaining a cell of a fish. In some embodiments, the method comprises providing or obtaining a cell of a bluefin tuna.

[069] In some embodiments, a fat accumulation inducing agent comprises: a free fatty acid or a free fatty acid and a peroxisome proliferator-activated receptor gamma (PPARγ) agonist.

[070] In some embodiments, a free fatty acid is in the form of an oil, free fatty acid mixture, or both. In some embodiments, an oil comprises flaxseed oil. In some embodiments, an oil comprises algae and/or microalgae oil.

[071] In some embodiments, a fat accumulation inducing agent consists essentially of a free fatty acid or a free fatty acid and a PPARγ agonist.

[072] As used herein, the term "consists essentially of" denotes that a given compound or substance, e.g., a free fatty acid or a free fatty acid and a PPARγ agonist, constitute(s) the vast majority of the active ingredient's e.g., fat accumulation inducing agent portion or fraction of the composition.

[073] In some embodiments, consists essentially of means that: a free fatty acid constitutes at least 95%, at least 98%, at least 99%, or at least 99.9% by weight, of the fat accumulation inducing agent of the composition, or any value and range therebetween. Each possibility represents a separate embodiment of the invention.

[074] In some embodiments, consists essentially of means that: a free fatty acid and a PPARγ agonist constitute at least 95%, at least 98%, at least 99%, or at least 99.9% by weight, of the fat accumulation inducing agent of the composition, or any value and range therebetween. Each possibility represents a separate embodiment of the invention.

[075] In some embodiments, a cell comprises a myoblast. In some embodiments, a cell comprises a fibroblast. In some embodiments, a cell comprises a combination of a myoblast and a fibroblast.

[076] In some embodiments, a free fatty acid comprises an unsaturated fatty acid. In some embodiments, the free fatty acid comprises an unsaturated fatty acid with 1 to 6 double bonds.

[077] In some embodiments, the free fatty acid comprises a fatty acid chain of up to 10, 12, 14, 16, 18, 20, 22, or 24 carbon atoms, or any value and range therebetween. Each possibility represents a separate embodiment of the invention. In some embodiments, the free fatty acid comprises a fatty acid chain of 10-24, 10-22, 10-20, 10-18, 12-18, 14-18, or 16-18 carbon atoms. Each possibility represents a separate embodiment of the invention.

[078] In some embodiments, the free fatty acid comprises a fatty acid chain of 10-22 carbon.

[079] In some embodiments, the free fatty acid comprises α-Linolenic acid. In some embodiments, the free fatty acid comprises oleic acid. In some embodiments, the free fatty acid comprises linoleic acid. In some embodiments, the free fatty acid comprises α-Linolenic acid and oleic acid. In some embodiments, the free fatty acid comprises α-Linolenic acid and linoleic acid. In some embodiments, the free fatty acid comprises oleic acid and linoleic acid. In some embodiments, the free fatty acid comprises α-Linolenic acid, oleic acid, and linoleic acid.

[080] In some embodiments, the free fatty acid comprises Eicosapentaenoic acid (EPA).

[081] In some embodiments, the free fatty acid comprises Docosahexaenoic acid (DHA).

[082] In some embodiments, the free fatty acid comprises one or more free fatty acids selected from: α-Linolenic acid, oleic acid, linoleic acid, EPA, and DHA.

[083] In some embodiments, the free fatty acid comprises at least one free fatty acid selected from: α-Linolenic acid, oleic acid, linoleic acid, EPA, DHA, and any combination thereof.

[084] The terms "oleic acid", "ethyl ricinoleate", "18:1-OH", "monounsaturated fatty acid" or "MUFA of 18 carbons" are used herein interchangeably.

[085] The terms "linoleic acid", "ethyl linoleate", and "18:2n6" are used herein interchangeably.

[086] The terms "α-Linolenic acid", "ethyl alpha linolenate", and "18:3n3" are used herein interchangeably.

[087] The terms "eicosapentaenoic acid", "EPA", and "20:5n3" are used herein interchangeably.

[088] The terms "docosahexaenoic acid", "DHA", and "22:6n3" are used herein interchangeably.

[089] In some embodiments, the composition comprises a free fatty acid in a concentration ranging between 50 μM and 350 μM, 50 μM and 300 μM, 50 μM and 250 μM, 50 μM and 200 μM, 50 μM and 150 μM, 50 μM and 100 μM, 100 μM and 350 μM, 150 μM and 350 μM, 200 μM and 350 μM, 250 μM and 350 μM, 150 μM and 300 μM, 200 μM and 300 μM, 150 μM and 250 μM, or 200 μM and 300 μM. Each possibility represents a separate embodiment of the invention.

[090] As used herein, the term "PPARγ agonist" refers to any compound that is capable of binding and/or activating the peroxisome proliferator-activated receptor. In some embodiments, the PPARγ agonist is a non-toxic PPARγ agonist. In some embodiments, the PPARγ agonist is not toxic to a cell obtained or derived from a bluefin tuna. In some embodiments, toxicity is determined according to any common method known to a person of skill in the art, such as, but not limited to, the means exemplified herein. In some embodiments, the PPARγ agonist being toxic to a cell of a bluefin tuna, is not toxic to a control cell. In some embodiments, a control cell comprises a fibroblast, a myoblast, or both, being derived from a non-bluefin tuna organism (or a cell line derived therefrom). In some embodiments, a control cell comprises or is a myoblast of a mackerel, yellowtail fish (also known as "Great Amberjack"), or both. In some embodiments, a control cell comprises or is an adipogenic progenitor cell. In some embodiments, an adipogenic progenitor control is obtained or derived from bovine.

[091] Non-limiting examples of yellowtail fish include, but are not limited to, Atlantic bumper (*Chloroscombrus chrysurus*); Yellowtail flounder (*Limanda ferruginea*); Yellowtail snapper (*Ocyurus chrysurus*); Whitespotted devil (*Plectroglyphidodon lacrymatus*); and Yellowtail horse mackerel (*Trachurus novaezelandiae*), to name a few.

[092] Methods and means for determining that the a given compound is a PPARγ agonist, are common and would be apparent to one of ordinary skill in the art. Non-limiting examples for such methods and/or means can be found in Christensen and El-Houri ("Development of an In Vitro Screening Platform for the Identification of Partial PPARγ Agonists as a Source for Antidiabetic Lead Compounds"; Molecules (2018) 23, 2431.

[093] In some embodiments, the PPARγ agonist comprises or is Indomethacin. In some embodiments, the PPARγ agonist comprises or is Rosiglitazone. In some embodiments, the PPARγ agonist comprises or is L-α-Phosphatidylcholine. In some embodiments, the PPARγ agonist comprises or is Indomethacin and Rosiglitazone. In some embodiments, the PPARγ agonist comprises or is Rosiglitazone and L-α-Phosphatidylcholine. In some embodiments, the PPARγ agonist comprises or is Indomethacin, Rosiglitazone, and L-α-Phosphatidylcholine. In some embodiments, the PPARγ agonist is in the form of a plant material comprising a PPARγ agonist. In some embodiments, the plant material comprises an extract, homogenate, isolate, any fraction thereof, or any combination thereof, being derived from a plant. In some embodiments, the plant is selected from: soy, sunflower, or a combination thereof. In some embodiments, the PPARγ agonist is a synthetically synthesized PPARγ agonist. In some embodiments, the PPARγ agonist is derived from a natural source, such as, but not limited to a plant material. In some embodiments, the PPARγ agonist is the form of lecithin comprising the PPARγ agonist. In some embodiments, the PPARγ agonist is or comprises lecithin comprising the PPARγ agonist.

[094] In some embodiments, the PPARγ agonist is not isobutyl-1-methylxanthine (IBMX). In some embodiments, the PPARγ agonist excludes IBMX.

[095] In some embodiments, the composition comprises Indomethacin in a concentration ranging between 1 nM and 10 nM, 1 nM and 8 nM, 1 nM and 6 nM, 2 nM and 10 nM, 4 nM and 10 nM, 3 nM and 7 nM, 2 nM and 9 nM, or 4 nM and 6 nM. Each possibility represents a separate embodiment of the invention.

[096] In some embodiments, the composition comprises Rosiglitazone in a concentration ranging between 5 μM and 20 μM, 5 μM and 18 μM, 5 μM and 15 μM, 5 μM and 13 μM, 6 μM and 20 μM, 7 μM and 18 μM, 7 μM and 15 μM, 6 μM and 12 μM, or 8 μM and 12 μM. Each possibility represents a separate embodiment of the invention.

[097] In some embodiments, the composition comprises L-α-Phosphatidylcholine in a concentration ranging between 1 ng/ml and 20 ng/ml, 1 ng/ml and 15 ng/ml, 1 ng/ml and 12

ng/ml, 3 ng/ml and 20 ng/ml, 3 ng/ml and 15 ng/ml, 5 ng/ml and 15 ng/ml, 6 ng/ml and 13 ng/ml, or 8 ng/ml and 12 ng/ml. Each possibility represents a separate embodiment of the invention.

[098] In some embodiments, the composition comprises Indomethacin and Rosiglitazone, each in a concentration as disclosed herein. In some embodiments, the composition comprises Indomethacin and L-α-Phosphatidylcholine, each in a concentration as disclosed herein. In some embodiments, the composition comprises Rosiglitazone and L-α-Phosphatidylcholine, each in a concentration as disclosed herein. In some embodiments, the composition comprises Indomethacin, Rosiglitazone, and L-α-Phosphatidylcholine, each in a concentration as disclosed herein.

[099] In some embodiments, the fat accumulation inducing agent consists essentially of α-Linolenic acid in a concentration ranging between 100 μM and 300 μM in the composition. In some embodiments, the fat accumulation inducing agent consists essentially of: α-Linolenic acid in a concentration ranging between 100 μM and 300 μM in the composition, and Rosiglitazone in a concentration ranging between 7 μM and 15 μM in the composition.

[0100] In some embodiments, the fat accumulation inducing agent consists essentially of oleic acid in a concentration ranging between 100 μM and 300 μM in the composition. In some embodiments, the fat accumulation inducing agent consists essentially of oleic acid in a concentration ranging between 100 μM and 300 μM in the composition, and Rosiglitazone in a concentration ranging between 7 μM and 15 μM in the composition.

[0101] In some embodiments, the composition is devoid of: 3-isobutyl-1-methylxanthine (IBMX). In some embodiments, the composition is devoid of insulin. In some embodiments, the composition is devoid of dexamethasone. In some embodiments, the composition is devoid of IBMX, insulin, dexamethasone, or any combination thereof.

[0102] In some embodiments, the method further comprises contacting a fish cell with an effective amount of a fibroblast growth factor (FGF). In some embodiments, FGF is or comprises fish FGF. In some embodiments, FGF is or comprises salmon FGF. In some embodiments, FGF is or comprises tuna FGF. In some embodiments, FGF comprises or is basic FGF (bFGF). In some embodiments, FGF is or comprises FGF2. In some embodiments, an effective amount of salmon FGF comprises FGF in a concentration of 0.1 ng/ml to 50 ng/ml, 1 ng/ml to 50 ng/ml, 5 ng/ml to 50 ng/ml, 10 ng/ml to 50 ng/ml, 20 ng/ml to 50 ng/ml, 15 ng/ml to 40 ng/ml, 10 ng/ml to 30 ng/ml, 15 ng/ml to 25 ng/ml, 20 ng/ml to 30 ng/ml. Each possibility

represents a separate embodiment of the invention. In some embodiments, a concentration of FGF refers to the final concentration of FGF in the cell culture medium wherein the fish cell is cultured.

[0103] In some embodiments, the cultured fish cell is an induced fish cell. In some embodiments, the cultured fish cell is an induced cultured fish cell. In some embodiments, indued is or comprises induced to accumulate fat.

[0104] In some embodiments, the method further comprises culturing a fish cell after the contacting (step). In some embodiments, culturing is in a suitable cell culture medium.

[0105] In some embodiments, contacting, culturing, or both, according to the method of the invention is in a temperature ranging between 18 °C and 26 °C, 19 °C and 25 °C, 20 °C and 21 °C, 20 °C and 22 °C, 20 °C and 23 °C, 20 °C and 24 °C, or 19 °C and 24 °C. Each possibility represents a separate embodiment of the invention.

[0106] In some embodiments, the composition comprises is "serum-free" or "animal-free media" composition.

[0107] In some embodiments, a fish is or comprises a tuna fish. In some embodiments, a tuna fish is or comprises a bluefin tuna fish.

[0108] In some embodiments, a bluefin tuna fish comprises: Atlantic bluefin tuna fish, Pacific bluefin tuna fish, Southern bluefin tuna fish, or any combination thereof.

[0109] In some embodiments, a bluefin tuna belongs to the genus *Thunnus*.

[0110] In some embodiments, a bluefin tuna is of or belongs to a species selected from: *T. maccoyii*, *T. orinetalis*, *T. thynnus*, and *T. tonggol*.

[0111] In some embodiments, a fish is selected from: Atlantic salmon (*Salmo salar*), Pacific salmon species including *Coho salmon* (*Oncorhynchus kisutch*), Chinook salmon (*Oncorhynchus tshawytscha*), Atlantic mackerel (*Scomber scombrusi*t), Pacific mackerel (*Scomber japonicus*), European pilchard (*Sardina pilchardus*), commonly referred to as sardines, Atlantic herring (*Clupea harengus*), Pacific herring (*Clupea pallasii*), Rainbow trout *(Oncorhynchus mykiss)*, Atlantic halibut (*Hippoglossus hippoglossus*), Pacific halibut (*Hippoglossus stenolepis*), European anchovy (*Engraulis encrasicolus*), Arctic Char (*Salvelinus alpinus*), Japanese eel (*Anguilla japonica*), European eel (*Anguilla anguilla*), Atlantic cod (*Gadus morhua*), Pacific cod (*Gadus macrocephalus*), Alaska pollock (*Gadus chalcogrammus*), Common dolphinfish, also known as Mahi-Mahi (*Coryphaena hippurus*),

Striped bass (*Morone saxatilis*), European sprat (*Sprattus sprattus*), Atlantic halibut (*Hippoglossus hippoglossus*), Pacific halibut (*Hippoglossus stenolepis*), Greenland halibut (*Reinhardtius hippoglossoides*), Swordfish (*Xiphias gladius*), or any combination thereof.

[0112] In some embodiments, the method is an ex vivo or an in vitro method. In some embodiments, the method is performed *ex vivo* or *in vitro*.

[0113] A person of skill in the art would acknowledge that ex vivo and/or in vitro methods/assays are performed in plate, a tube, or any equivalent thereof. In some embodiments, the method is performed in plate, a tube, or any equivalent thereof. In some embodiments, the method is not performed in the body of an organism, e.g., in vivo. In some embodiments, the method is not performed in vivo.

[0114] According to another aspect, there is provided a cell obtained or derived from a bluefin tuna, wherein the cell being induced to accumulate fat according to the method of the invention.

[0115] According to another aspect, there is provided a composition comprising the cell of the invention. In some embodiments, the composition further comprises an acceptable carrier.

[0116] In some embodiments, the carrier is a nutraceutical carrier. In some embodiments, the composition is a nutraceutical composition. In some embodiments, the composition is an edible composition.

[0117] As used herein, the phrase "a nutraceutical acceptable carrier, diluent or excipient" refers to any adjuvant, carrier, excipient, glidant, sweetener, diluent, preservative, dye/colorant, flavor enhancer, surfactant, wetting agent, dispersing agent, suspending agent, stabilizing agent, isotonic agent, solvent, or emulsifying agent.

[0118] In some embodiments, the composition is a pre-mix composition. In some embodiments, a pre-mix composition comprises or is an ingredient to be further mixed with at least one additional ingredient so as to form a product. In some embodiments, the product is an edible product. In some embodiments, the product is a foodstuff.

[0119] According to another aspect, there is provided a food product, comprising the cell obtained according to the method of the invention, the composition of the invention, or both.

[0120] In some embodiments, a "nutraceutical composition" refers to a preparation of a composition as described herein with other chemical components such as physiologically suitable carriers and excipients. The purpose of a nutraceutical composition is to facilitate administration of the composition to an organism. In one embodiment, the phrases

"physiologically acceptable carrier" refer to a carrier or a diluent that does not cause significant irritation to a mammal and does not abrogate the biological activity and properties of the administered composition.

[0121] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[0122] As used herein, the term "about" when combined with a value refers to plus and minus 10% of the reference value. For example, a length of about 1,000 nanometers (nm) refers to a length of 1,000 nm ± 100 nm.

[0123] It is noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a polynucleotide" includes a plurality of such polynucleotides and reference to "the polypeptide" includes reference to one or more polypeptides and equivalents thereof known to those skilled in the art, and so forth. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements or use of a "negative" limitation.

[0124] In those instances where a convention analogous to "at least one of A, B, and C, etc." is used, in general such a construction is intended in the sense one having skill in the art would understand the convention (e.g., "a system having at least one of A, B, and C" would include but not be limited to systems that have A alone, B alone, C alone, A and B together, A and C together, B and C together, and/or A, B, and C together, etc.). It will be further understood by those within the art that virtually any disjunctive word and/or phrase presenting two or more alternative terms, whether in the description, claims, or drawings, should be understood to contemplate the possibilities of including one of the terms, either of the terms, or both terms. For example, the phrase "A or B" will be understood to include the possibilities of "A" or "B" or "A and B."

[0125] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the embodiments pertaining to the invention are specifically embraced by the present invention and are disclosed herein just as if each and every combination was individually and explicitly disclosed. In addition, all sub-combinations of the various embodiments and elements thereof are also specifically embraced by the present invention and are disclosed herein just as if each and every such sub-combination was individually and explicitly disclosed herein.

[0126] Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

[0127] Various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below find experimental support in the following examples.

EXAMPLES

[0128] Generally, the nomenclature used herein, and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological, and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Culture of Animal Cells - A Manual of Basic Technique" by Freshney, Wiley-Liss, N. Y. (1994), Third Edition; "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton &

Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference. Other general references are provided throughout this document.

EXAMPLE 1

[0129] Intramuscular fat (IMF) corresponds to the amount of fat within muscles. The notion is that IMF content positively influences fish's sensory quality (e.g., taste, flavor, aroma, and firmness). The inventors aimed to develop a method for IMF and fat accumulation (FA) in Bluefin Tuna (BFT) cells. Previous works examined fatty acid accumulation during the differentiation of fish pre-adipocyte cells and with fish myoblast cells (Salmerón et al., 2013; Saad et al., 2023), and showed potential fatty acid accumulation in Mackerel myoblast cells. In this previous study, Mackerel myoblast cells undergone FA within seven days. Mackerel myoblast cells were treated with 3-isobutyl-1-methylxanthine (IBMX), a PPAR gamma agonist, and human insulin, with the addition of dexamethasone and lipid mixture (a combination of animal-derived lipids from cod liver; Sigma Aldrich #L5146). In the current study, the inventors applied a similar method in the attempt to induce FA and IMF in Mackerel, Yellowtail, and BFT cells. This method successfully led to FA in Mackerel myoblasts and BFT fibroblasts. However, when BFT myoblasts were treated similarly, severe cell toxicity was observed (**Fig. 1**). The inventors examined which component in the mix was responsible for causing cell death and found that either human insulin at 10 µg/ml or the lipid mixture at 1:100 dilution was effectively causing the toxic effect (**Fig. 1B**). The inventors found that this deleterious effect is relieved when these components are further tenfold diluted (e.g., 1 µg/ml for human insulin and 1:1,000 for the lipid mixture). However, no FA phenotype was observed for the diluted mixture when applied according to the FA protocol (IDIL reduced; **Fig. 1B**).

[0130] The inventors further tested different methods in the attempt to induce FA in BFT, such as according to previously described publications (Caponi et al., 2023). Specifically, Caponi et al., used pre-adipocytic bovine cells for FA and applied Indomethacin (a PPAR gamma agonists) and a chemically defined lipid concentrate (CDLC; primarily free fatty acids; Thermo Fisher #11905031) as the lipid source. Caponi et al., observed FA in pre-adipocytic bovine cells eight days post treatment. When applying the CDLC as a lipid source, together with Indomethacin or Rosiglitazone to BFT cells, according to the teachings of Caponi et al., (as well as of Mitić et al., 2022), the current inventors observed underwhelming results, with no FA in either BFT fibroblasts or BFT myoblasts (**Fig. 2A**).

[0131] Previous efforts for FA included activation of the nuclear hormone receptor PPAR gamma in the presence of different fatty acid sources, in the attempt to induce lipid droplet accumulation within the cell cytoplasm. For example, Pasitka et al., 2023 used Rosiglitazone or L-α-Phosphatidylcholine as PPAR gamma agonists and oleic acid as a lipid source to induce FA in chicken fibroblasts in seven days. In efforts to develop a protocol that allows FA and IMF formation in BFT cells, the inventors have screened different combinations of PPAR gamma agonists and fatty acids that may meet the specific requirements of BFT cells for FA, and importantly, such combination that are not toxic to the cells.

[0132] BFT myoblasts were grown in media containing L-15 medium. Since toxicity in BFT cells was observed in the inventors' initial efforts to induce FA in these cells, the inventors started by mapping the toxicity levels of the PPAR gamma agonists. The inventors have tested three different PPAR gamma agonists – namely, Rosiglitazone (a specific PPARγ agonist of the thiazolidinedione family), Indomethacin (a nonsteroidal anti-inflammatory drug; NSAID;), and L-α-Phosphatidylcholine (found typically in raw soy and other plants' lecithin). The inventors first tested whether each of these agonists may be toxic to BFT myoblast cells. The inventors treated the cells with increasing concentrations of the afore-mentioned agonists for up to 11 days, with either: (i) 5 or 10 μM Rosiglitazone; (ii) 5, 10, or 25 nM Indomethacin; or (iii) 1, 5, 10, or 20 ng/ml L-α-Phosphatidylcholine. Cell viability was monitored using the PrestoBlue viability assay (a colorimeter bioassay) and under microscopy. Healthy cells, as well as no cell toxicity, were observed under any of the tested concentrations (**Figs. 2B-2C**). Next, the inventors tested whether PPARγ agonists alone are sufficient to induce FA in BFT cells. The examined concentrations were chosen based on previous reports (Caponi et al., 2023; Pasitka et al., 2023). The inventors found that treatment with PPARγ agonist alone has no effect on FA in BFT fibroblasts (**Fig. 2D**). When testing the effect of treatment with PPARγ agonist alone in BFT myoblasts, minor levels of FA were observed. Treating BFT myoblasts exclusively with indomethacin, L-α-Phosphatidylcholine, or Rosiglitazone resulted with induction of FA at a level of about 40%, 19%, or 16%, respectively (**Fig. 2E**). The inventors concluded that these modest levels of FA were likely obtained due to the availability of free fatty acids being present in the serum of the culture media.

[0133] Next, the inventors screened for a combination of a PPAR gamma agonist and free fatty acids that could lead to FA and IMF in BFT myoblasts. Different types and concentrations of PPAR gamma agonist and free fatty acids were supplemented to the cells in the growth media, and FA levels were determined. To induce FA in BFT myoblasts, the media was

supplemented with the addition of the combination of PPAR gamma agonist and free fatty acid source/s supplemented with specific concentrations. Cells were cultured and monitored for FA and IMF for three days. To monitor FA, cells were evaluated by morphological changes, e.g., formation of lipid droplets in the cytoplasm. Specifically, IF staining and visualization for the neutral lipid dyes BODIPY 493/503 (Thermo Fisher #D3922), or Nile Red stains (Sigma #72485), Flow Cytometry analysis for neutral lipid accumulation (using BODIPY or Nile Red), and quantification of triglyceride formation assay (Cayman #10010303), were applied.

[0134] The inventors have screened three different PPAR gamma agonists: Rosiglitazone (5 μM-10 μM), Indomethacin (5 nM), and L-α-Phosphatidylcholine (12 ng/ml). The inventors also screened multiple sources of lipid mixtures and free fatty acids (See **Table 1** for the list of FA treatment combinations). Treating BFT myoblasts with commercial lipid mixtures CDLC, Thermo Fisher #11905031; Lipid Mixture, Sigma #L5146; Intralipid, Sigma #I141) did not induce FA. Furthermore, treating BFT myoblasts with only PPAR gamma agonists (e.g., without a fatty acid source) did not induce FA. Palmitic acid, stearic acid, and arachidonic acid did not induce FA, and in fact were toxic to BFT myoblasts. In sharp contrast to the above fatty acids, surprisingly, linoleic acid, oleic acid, and alpha linolenic acid, had successfully induced FA while not compromising cell viability. Oleic acid (OA), a monounsaturated omega-9 fatty acid, and alpha-linolenic acid (a-LA) a polyunsaturated omega-3 fatty acid and a precursor of long-chain polyunsaturated omega-3 fatty acids, were shown to have a pronounced FA induction effect.

[0135] When the inventors treated BFT myoblasts with a combination comprising 200 μM OA and individual PPARγ agonists (as disclosed hereinabove), FA was observed in all treatments. To this end, all three agonists successfully induced lipid droplet formation (**Fig. 3A**). The combination of Rosiglitazone with OA presented a pronounced effect. FACS analysis showed that 20% of BFT myoblasts were positive for BODIPY when treated with 12 μg/ml L-α-Phosphatidylcholine and 300 μM OA for three days, 48% of BFT myoblasts were positive for BODIPY when treated with 5 nM Indomethacin and 300 μM OA for a similar period, 23% of BFT myoblasts were positive for BODIPY following a 10 μM Rosiglitazone + 200 μM OA treatment, and 65% of BFT myoblasts were positive for BODIPY following a 10 μM Rosiglitazone + 300 μM OA treatment (**Fig. 3B**).

[0136] Free fatty acids are known to be esterified with glycerol, and subsequently stored as triglycerides within the cell cytoplasm. Quantifying the levels of fatty acids as triglycerides in 10 μM Rosiglitazone + 300 μM OA treated myoblasts showed a gradual increase in the level

of triglycerides along the three days of FA treatment (**Fig. 3C**). The results indicate that a combination of OA and the PPARγ agonist Rosiglitazone provides a strong FA effect to this extent, in BFT myoblasts.

[0137] Further, the inventors tested the effect of Rosiglitazone on FA while using other sources of free fatty acids. The inventors observed FA in BFT myoblasts treated with 10 μM Rosiglitazone and 300 μM aLA. This treatment led to the accumulation of lipid droplets within the cell cytoplasm within three days. FACS studies for BODIPY staining showed induction of FA in 94.35% of BFT myoblasts (**Fig. 3B**) as early as three days after FA treatment. Quantifying the levels of fatty acids as triglycerides showed an increase in the level of triglycerides, as well, three days after FA treatment (**Fig. 3D**). The results indicate that Rosiglitazone and 300 μM a-LA as the source of fatty acids leads to an enhanced FA outcome in BFT myoblasts.

[0138] Corresponding to the current approach with BFT myoblasts, the inventors examined the conditions for FA in BFT fibroblasts (See **Table 2** for the list of FA treatment combinations). To induce FA, a combination of PPAR gamma agonists and fatty acids were added to the media in specific concentrations, and cells were evaluated for FA for three days. When treated with each PPARγ agonist with 300 μM OA, BFT fibroblasts undergone FA in all treatments. FA in BFT fibroblasts differs from that observed in BFT myoblasts (**Fig. 4A**). The formation of lipid droplets in the cytoplasm appears as punctate dots in both cell lines. However, following FA, the fibroblasts have a round and flattened morphology, whereas the myoblast cells retain an elongated cell morphology. Additionally, in BFT fibroblasts, the lipid droplets seem more prominent than the lipid droplets in BFT myoblast cells. While all three agonists successfully induced lipid droplet formation, the combination of 10 μM Rosiglitazone and 300 μM OA presents a pronounced effect. FACS studies for BODIPY staining showed the induction of FA. After three days of FA treatment, 27% of the cells were positive for BODIPY when treated with 12 μg/ml L-α-Phosphatidylcholine and 300 μM OA, 50% were positive when treated with 5 nM Indomethacin and 300 μM OA, and 71% of BFT fibroblasts were positive for BODIPY following the 10 μM Rosiglitazone + 300 μM OA treatment (**Fig. 4B**). Next, the inventors tested the combination of treatments with Rosiglitazone together with aLA. High FA levels were observed when BFT fibroblasts were treated with 10 μM Rosiglitazone and 300 μM aLA (**Fig. 4A**). Following three days of treatment, the fibroblasts were analyzed by FACS for BODIPY staining. Analysis showed that 99% of fibroblasts treated with 10 μM Rosiglitazone and 300 μM aLA were positive for BODIPY compared to 71% when treated with 10 μM Rosiglitazone and 300 μM OA (**Fig. 4B**). Finally, quantifying the levels of triglycerides

showed an increase in triglycerides after the three days of FA treatment in both combinations of treatments (Rosiglitazone + OA and Rosiglitazone + aLA; **Fig. 4C**). These results indicate that combining Rosiglitazone as a PPARγ agonist and 300 µM aLA as the source of fatty acids substantially increase FA in BFT fibroblasts.

[0139] Unpredictably, when BFT cells were treated with a fatty acid source alone (either OA or a-LA), that is in the absence of PPARγ agonist, FA was observed. BFT myoblasts treated with 200-300 µM OA or 200-300 µM aLA presented accumulation of lipid droplets within the cell cytoplasm, as early as after three days of treatment. FACS analysis for BODIPY showed that 15.81%, 59.28%, 37.72%, and 94.69% of BFT myoblasts presented FA when treated with 200 µM OA, 300 µM OA, 200 µM aLA, and 300 µM aLA, respectively, as early as after three days of treatment (**Fig. 5A**). Similarly, the levels of triglycerides showed an increase over the three days of treatment (**Fig. 5B**). Similarly to myoblasts, when the inventors tested FA with only fatty acid source in BFT fibroblasts, BODIPY analysis showed that 37.35%, and 69% of BFT fibroblasts presented FA when treated with 300 µM OA, and 300 µM aLA, respectively (Figs. **5C-5D**). Quantifying the levels of fatty acids as triglycerides with 300 µM of either OA or aLA treated fibroblasts showed increased triglycerides along the three days of FA treatment (**Fig. 5E**). The current study indicates that BFT cells can be manipulated to accumulate fat provided as a fatty acid source either in the absence or presence of a PPARγ agonist.

Table 1. Fat Accumulation in BFT myoblasts – Summary

PPARγ Agonist	Lipid source	Result
5 µM Rosiglitazone	None	No lipid droplet formation
10 µM Rosiglitazone	None	No lipid droplet formation
10 µM Rosiglitazone	0.1% CDLC (Thermo fisher 11905031)	No lipid droplet formation
	1% CDLC (Thermo fisher 11905031)	No lipid droplet formation
	0.1% Lipid Mixture (Sigma L5146)	No lipid droplet formation

	1% Lipid Mixture (Sigma L5146)	No lipid droplet formation
	100 ng/ml Intralipid (Sigma I141)	No lipid droplet formation
	500 ng/ml Intralipid (Sigma I141)	No lipid droplet formation
	1 µg/ml Intralipid (Sigma I141)	No lipid droplet formation
	1.5 µg/ml Intralipid (Sigma I141)	No lipid droplet formation
	100 µM Oleic acid	Lipid droplets visible in cytoplasm
	200 µM Oleic acid	Lipid droplets visible in cytoplasm
	300 µM Oleic acid	Lipid droplets visible in cytoplasm
	400 µM Oleic acid	Toxic
	50 µM Palmitic acid	Toxic
	100 µM Palmitic acid	Toxic
	200 µM Palmitic acid	Toxic
	200 µM Stearic acid	Toxic
	300 µM Stearic acid	Toxic
	100 µM Arachidonic acid	Toxic
	200 µM Arachidonic acid	Toxic
	300 µM Arachidonic acid	Toxic
	100 µM α-Linolenic acid	Lipid droplets visible in cytoplasm
	200 µM α-Linolenic acid	Lipid droplets visible in cytoplasm
	300 µM α-Linolenic acid	Lipid droplets visible in cytoplasm

	400 µM µ-Linolenic acid	Toxic
	100 µM Linoleic Acid (Sigma L5900)	Lipid droplets visible in cytoplasm
	200 µM Linoleic Acid (Sigma L5900)	Lipid droplets visible in cytoplasm
	300 µM Linoleic Acid (Sigma L5900)	Lipid droplets visible in cytoplasm
	100 µM DHA	Small amounts of lipid droplets visible
	200 µM DHA	Small amounts of lipid droplets visible
	300 µM DHA	Toxic
	200 µM DPA	Toxic
5 nM Indomethacin	100 µM Oleic acid	Lipid droplets visible in cytoplasm
	200 µM Oleic acid	Lipid droplets visible in cytoplasm
	300 µM Oleic acid	Lipid droplets visible in cytoplasm
	100 µM Linoleic Acid (Sigma L5900)	Lipid droplets visible in cytoplasm
	200 µM Linoleic Acid (Sigma L5900)	Lipid droplets visible in cytoplasm
	300 µM Linoleic Acid (Sigma L5900)	Lipid droplets visible in cytoplasm
	50 µM Palmitic acid	Toxic
	100 µM Palmitic acid	Toxic
	200 µM Palmitic acid	Toxic
12 ng/ml L-α-Phosphatidylcholine	100 µM Oleic acid	Lipid droplets visible in cytoplasm
	200 µM Oleic acid	Lipid droplets visible in cytoplasm

	300 µM Oleic acid	Lipid droplets visible in cytoplasm
	100 µM-300 µM α-Linolenic acid	Lipid droplets visible in cytoplasm
	100 µM Linoleic Acid (Sigma L5900)	Lipid droplets visible in cytoplasm
	200 µM Linoleic Acid (Sigma L5900)	Lipid droplets visible in cytoplasm
	300 µM Linoleic Acid (Sigma L5900)	Lipid droplets visible in cytoplasm
	50 µM Palmitic acid	Toxic
	100 µM Palmitic acid	Toxic
	200 µM Palmitic acid	Toxic
	100 µM α-Linolenic acid	Lipid droplets visible in cytoplasm
	200 µM α-Linolenic acid	Lipid droplets visible in cytoplasm
	300 µM α-Linolenic acid	Lipid droplets visible in cytoplasm
	200 µM DHA	Small amounts of lipid droplets visible

Table 2. Fat Accumulation in BFT fibroblast – Summary

PPARγ Agonist	Lipid scours	Result
10 µM Rosiglitazone	0.1% CDLC (Thermo fisher 11905031)	No lipid droplet formation
	1% CDLC (Thermo fisher 11905031)	No lipid droplet formation
	0.1% Lipid Mixture (Sigma L5146)	No lipid droplet formation
	1% Lipid Mixture (Sigma L5146)	No lipid droplet formation

	100 ng/ml Intralipid (Sigma I141)	No lipid droplet formation
	500 ng/ml Intralipid (Sigma I141)	No lipid droplet formation
	1 µg/ml Intralipid (Sigma I141)	No lipid droplet formation
	1.5 µg/ml Intralipid (Sigma I141)	No lipid droplet formation
	100 µM Oleic acid	Lipid droplets visible in cytoplasm
	200 µM Oleic acid	Lipid droplets visible in cytoplasm
	300 µM Oleic acid	Lipid droplets visible in cytoplasm
	400 µM Oleic acid	Lipid droplets visible in cytoplasm
	50uM Palmitic acid	Toxic
	100 µM Palmitic acid	Toxic
	200 µM Palmitic acid	Toxic
	200 µM Stearic acid	Toxic
	300 µM Stearic acid	Toxic
	100 µM Arachidonic acid	Toxic
	200 µM Arachidonic acid	Toxic
	300 µM Arachidonic acid	Toxic
	100 µM α-Linolenic acid	Lipid droplets visible in cytoplasm
	200 µM α -Linolenic acid	Lipid droplets visible in cytoplasm
	300 µM α -Linolenic acid	Lipid droplets visible in cytoplasm
	400 µM α -Linolenic acid	Lipid droplets visible in cytoplasm

	100 µM Linoleic Acid (Sigma L5900)	Lipid droplets visible in cytoplasm
	200 µM Linoleic Acid (Sigma L5900)	Lipid droplets visible in cytoplasm
	300 µM Linoleic Acid (Sigma L5900)	Lipid droplets visible in cytoplasm
	400 µM Linoleic Acid (Sigma L5900)	Lipid droplets visible in cytoplasm
	100 µM DHA	Small amounts of lipid droplets visible
	200 µM DHA	Toxic
	300 µM DHA	Toxic
	100 µM DPA	Small amounts of lipid droplets visible
5 nM Indomethacin	0.1% CDLC (Thermo fisher 11905031)	No lipid droplet formation
	1% CDLC (Thermo fisher 11905031)	No lipid droplet formation
	0.1% Lipid Mixture (Sigma L5146)	No lipid droplet formation
	1% Lipid Mixture (Sigma L5146)	No lipid droplet formation
	100 µM Oleic acid	Lipid droplets visible in cytoplasm
	200 µM Oleic acid	Lipid droplets visible in cytoplasm
	300 µM Oleic acid	Lipid droplets visible in cytoplasm
	100 µM Linoleic Acid (Sigma L5900)	Lipid droplets visible in cytoplasm
	200 µM Linoleic Acid (Sigma L5900)	Lipid droplets visible in cytoplasm
	300 µM Linoleic Acid (Sigma L5900)	Lipid droplets visible in cytoplasm

	50 µM Palmitic acid	Toxic
	100 µM Palmitic acid	Toxic
	200 µM Palmitic acid	Toxic
12 ng/ml L-α-Phosphatidylcholine	0.1% CDLC (Thermo fisher 11905031)	No lipid droplet formation
	1% CDLC (Thermo fisher 11905031)	No lipid droplet formation
	0.1% Lipid Mixture (Sigma L5146)	No lipid droplet formation
	1% Lipid Mixture (Sigma L5146)	No lipid droplet formation
	100 µM Oleic acid	Lipid droplets visible in cytoplasm
	200 µM Oleic acid	Lipid droplets visible in cytoplasm
	300 µM Oleic acid	Lipid droplets visible in cytoplasm
	100 µM-300 µM α -Linolenic acid	Lipid droplets visible in cytoplasm
	100 µM Linoleic Acid (Sigma L5900)	Lipid droplets visible in cytoplasm
	200 µM Linoleic Acid (Sigma L5900)	Lipid droplets visible in cytoplasm
	300 µM Linoleic Acid (Sigma L5900)	Lipid droplets visible in cytoplasm
	50 µM Palmitic acid	Toxic
	100 µM Palmitic acid	Toxic
	200 µM Palmitic acid	Toxic

Table 3. Fat Accumulation in BFT myoblasts with only a lipid source – Summary

Fatty Acid	Structure	Concentrations tested	Result

Oleic acid	C18:1	100 µM	Lipid droplets visible in cytoplasm
		200 µM	Lipid droplets visible in cytoplasm
		300 µM	Lipid droplets visible in cytoplasm
		400 µM	Toxic
Stearic acid	C18:0	200 µM	Toxic
		300 µM	Toxic
α-Linolenic acid	C18:3 (n-3)	100 µM	Lipid droplets visible in cytoplasm
		200 µM	Lipid droplets visible in cytoplasm
		300 µM	Lipid droplets visible in cytoplasm
		400 µM	Toxic
Linoleic Acid (Sigma L5900)	C18:2 (n-6)	100 µM	Lipid droplets visible in cytoplasm
		200 µM	Lipid droplets visible in cytoplasm
		300 µM	Lipid droplets visible in cytoplasm
Arachidonic acid	C20:4 (n-6)	200 µM	Toxic
		300 µM	Toxic

Table 4. Fat Accumulation in BFT fibroblasts with only a lipid source – Summary

Fatty Acid	Structure	Concentrations tested	Result
Oleic acid	C18:1	100 µM	Lipid droplets visible in cytoplasm
		200 µM	Lipid droplets visible in cytoplasm
		300 µM	Lipid droplets visible in cytoplasm
		400 µM	Lipid droplets visible in cytoplasm
Stearic acid	C18:0	200 µM	Toxic
		300 µM	Toxic

α -Linolenic acid	C18:3 (n-3)	100 µM	Lipid droplets visible in cytoplasm
		200 µM	Lipid droplets visible in cytoplasm
		300 µM	Lipid droplets visible in cytoplasm
		400 µM	Lipid droplets visible in cytoplasm
Linoleic Acid (Sigma L5900)	C18:2 (n-6)	100 µM	Lipid droplets visible in cytoplasm
		200 µM	Lipid droplets visible in cytoplasm
		300 µM	Lipid droplets visible in cytoplasm
		400 µM	Lipid droplets visible in cytoplasm
Arachidonic acid	C20:4 (n-6)	200 µM	Toxic
		300 µM	Toxic

EXAMPLE 2

[0140] Further to the above, the inventors examined the ability to induce fat in cells of other fish species, according to the method of the invention.

[0141] Corresponding to the current approach with the BFT myoblast and BFT fibroblast cells, the inventors examined the conditions for FA in Yellowtail/Greater amberjack (*Seriola dumerili*) fibroblast cells and in Tuna Albacore (*Thunnus alalunga*). The cells were grown in media containing L-15 medium, supplemented with 20% or 10% fetal bovine serum (FBS; for fibroblasts and myoblasts, respectively), 25 ng/ml salmon fibroblast growth factor (salmon FGF), 2% HEPES Buffer Solution (1 M), and 1% Penicillin/Streptomycin. To induce FA, Rosiglitazone, a PPAR gamma agonist and fatty acids were added to the media in specific concentrations, and cells were evaluated for FA for 24 hours. When treated with Rosiglitazone 10 µM with either alpha-linolenic acid (aLA; 200 or 300 µM) or Oleic acid (OA; 200 or 300 µM), the Yellowtail fibroblast cells undergone FA accumulation in all treatments. The formation of lipid droplets in the cytoplasm appeared as punctate dots with a round and flattened morphology. FA in Yellowtail fibroblast cells and in Tuna Albacore myoblast cells were

evaluated with BODIPY staining for neutral lipids, and nuclei were stained with DAPI (**Figs. 6A-6B**, respectively).

EXAMPLE 3

Material and Methods for the FAMEs Analysis

[0142] To induce FA, FBS concentration was lowered to 10%, 10 μM of Rosiglitazone and 300 μM aLA fatty acid were added to the media. Within 48 h lipid droplets were visible in fibroblast cytoplasm as punctate dots. After 48 h of FA treatment, cells were collected for FAMEs analysis.

Semiquantitative FAMEs Analysis

[0143] Fatty acid methyl esters (FAMEs) analysis serves as a method for identifying the characteristics of fats and oils and quantifying the total fat content in food samples. Fats are typically extracted using a nonpolar solvent, followed by saponification to convert them into free fatty acid salts. These free acids are then derivatized into methyl esters, which can be efficiently analyzed through gas chromatography.

[0144] The technique for qualitative and quantitative analysis of FAMEs used GC-FID equipped with the DB-23 column (Agilent), following transmethylation of the fatty acids in the samples (biomass or fish tissue). For the semi-quantitative analysis, an atypical fatty acid (C19:0) was added as an internal standard.

[0145] The FAME is provided in **Table 5** herein below, and **Figs. 7A-7B**.

Table 5. FAME analysis

	Fibroblasts				**Control**			
	Control Fibroblasts		Treat Fibroblasts		O-Toro		Akami	
	Weight %	% of total fatty acids	Weight %	% of total fatty acids	Weight %	% of total fatty acids	Weight %	% of total fatty acids

Saturated fatty acids	2.861	39.524	1.83	15.859	11.728	24.371	4.810	26.158
MUFA	1.622	22.402	0.828	7.181	19.883	41.318	7.855	42.718
PUFA-Omega 6 and Omega 9	1.65	16.094	0.824	7.144	1.124	2.336	0.400	2.174
PUFA-Omega 3	0.529	7.305	7.363	63.822	7.580	15.751	2.483	13.505
Unknown fatty acids	1.062	14.675	0.692	5.994	7.807	16.233	2.840	15.445
Total	7.239	100	11.536	100	48.121	100	18.389	100

[0146] The results show that cells induced to accumulate fat according to the claimed invention are characterized by increased amounts of polyunsaturated fatty acids, including omega-3, -6, and -9, and reduced amount of monounsaturated fatty acids, compared to non-induced cell control, and fibroblasts being derived from fresh tuna cuts, e.g., O-Toro and Akami controls.

EXAMPLE 4

[0147] The inventors further examine whether temperature range affects fat accumulation (FA) in bluefin tuna myoblasts.

[0148] The inventors grow the cells at standard growth conditions (20 °C) until confluency, then change to FA induction medium, and subject the cells to the following temperatures for 20-48 hours: 18 °C, 20 °C, 24 °C, 27 °C, 32 °C, or 37 °C.

[0149] The extent of fat accumulation is assessed by Bodipy staining and cell viability by Hoechst/DAPI staining. Fat accumulation cell viability is evaluated and compared to untreated cells and FA-treated cells in standard growth conditions (20 °C).

[0150] The inventors suggest that optimal fat accumulation takes place at 20-24 °C. Further, the inventors suggest that cell survival is compromised at 27 °C and above. Further, the inventors suggest that less efficient fat accumulation takes place at 18 °C or below.

[0151] Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications, and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

CLAIMS

1. A cultured fish cell comprising an increased amount of polyunsaturated fatty acids (PUFA) and reduced amount of monounsaturated fatty acids (MUFA) compared to a control fish cell.

2. The cultured fish cell of claim 1, wherein said control fish cell is derived from a wild caught or farm raised fish of the same species as said cultured fish cell.

3. The cultured fish cell of claim 1 or 2, wherein said polyunsaturated fatty acids comprise omega-3 PUFA, omega-6 PUFA, omega-9 PUFA, or any combination thereof.

4. The cultured fish cell of any one of claims 1 to 3, comprising omega-3 PUFA in an amount of 20% to 80% by weight of fatty acids in said cultured fish cell.

5. The cultured fish cell of any one of claims 1 to 4, being characterized by omega-3 PUFA to omega-6 PUFA weight per weight ratio (w/w) ranging between 8:1 (w/w) and 15:1 (w/w).

6. The cultured fish cell of any one of claims 1 to 5, wherein said cell is a fibroblast, a myoblast, or a combination thereof.

7. The cultured fish cell of any one of claims 1 to 6, wherein said fish belongs to the subfamily *Scombrinae*.

8. The cultured fish cell of any one of claims 1 to 7, wherein said fish belons to the genus *Thunnus*.

9. The cultured fish cell of claim 8, wherein said fish is of a species selected from the group consisting of: *T. maccoyii*, *T. orinetalis*, *T. thynnus*, *T. tonggol*, *T. alalunga*, and *T. albacares*.

10. A method for inducing fat accumulation in a fish cell, the method comprising contacting a fish cell with an effective amount of a composition comprising a fat accumulation inducing agent; and (ii) a carrier, wherein said fat accumulation inducing agent consists essentially of a free fatty acid and a peroxisome proliferator-activated receptor gamma (PPARγ) agonist, thereby inducing fat accumulation in the fish cell.

11. The method of claim 10, wherein said cell is a myoblast, a fibroblast, or a combination thereof.

12. The method of claim 10 or 11, wherein said free fatty acid is an unsaturated fatty acid.

13. The method of any one of claims 10 to 12, wherein said free fatty acid comprises a fatty acid chain of up to 18 carbon atoms.

14. The method of any one of claims 10 to 13, wherein said free fatty acid is selected from the group consisting of: α-Linolenic acid, oleic acid, linoleic acid, and any combination thereof.

15. The method of any one of claims 10 to 14, wherein said composition comprises said free fatty acid in a concentration ranging between 50 μM and 350 μM.

16. The method of any one of claims 10 to 15, wherein said PPARγ agonist is selected from the group consisting of: Indomethacin, Rosiglitazone, L-α-Phosphatidylcholine, and any combination thereof.

17. The method of claim 16, wherein said composition comprises any one of:

a. said Indomethacin in a concentration ranging between 1 nM and 10 nM;

b. said Rosiglitazone in a concentration ranging between 5 μM and 20 μM;

c. said L-α-Phosphatidylcholine in a concentration ranging between 1 ng/ml and 20 ng/ml; and

d. any combination of (a)-(c).

18. The method of any one of claims 10 to 17, wherein said fat accumulation inducing agent consists essentially of α-Linolenic acid in a concentration ranging between 100 μM and 300 μM in said composition, and Rosiglitazone in a concentration ranging between 7 μM and 15 μM in said composition.

19. The method of any one of claims 10 to 17, wherein said fat accumulation inducing agent consists essentially of oleic acid in a concentration ranging between 100 μM and 300 μM in said composition, and Rosiglitazone in a concentration ranging between 7 μM and 15 μM in said composition.

20. The method of any one of claims 10 to 19, wherein said composition is devoid of: 3-isobutyl-1-methylxanthine (IBMX), insulin, dexamethasone, or any combination thereof.

21. The method of any one of claims 10 to 20, wherein said fish cell is obtained or derived from a fish belonging to the subfamily *Scombrinae*.

22. The method of any one of claims 10 to 21, wherein said fish cell is obtained or derived from a fish being a tuna fish belonging to the genus *Thunnus*.

23. The method of claim 22, wherein said tuna fish is of a species selected from the group consisting of: *T. maccoyii*, *T. orinetalis*, *T. thynnus*, *T. tonggol*, *T. alalunga*, and *T. albacares*.

24. The method of any one of claims 10 to 23, further comprising contacting said fish cell with an effective amount of fibroblast growth factor (FGF).

25. The method of claim 24, wherein said FGF is salmon FGF.

26. The method of any one of claims 10 to 25, further comprising culturing said fish cell after said contacting.

27. The method of claim 26, wherein said contacting, culturing, or both, is in a temperature ranging between 18 °C and 26 °C.

28. The method of any one of claims 10 to 27, being an ex vivo or an in vitro method.

29. A composition comprising the cultured fish cell of any one of claims 1 to 9, being an edible composition.

30. A food product, comprising the composition of claim 29.