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Review

Promises of Nanotherapeutics in Obesity

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The application of nanotechnology to medicine promises a wide range of new tools and possibilities, from earlier diagnostics and improved imaging, to better, more efficient, and more targeted therapies. This emerging field could help address obesity, with advances in drug delivery, nutraceuticals, and genetic and epigenetic therapeutics. Its application to obesity is still largely in the development phase. Here, we review the novel angle of nanotech applied to human consumable products and their specific applications to addressing obesity through nutraceuticals, with respect to benefits and limitations of current nanotechnology methods. Further, we review potential future applications to deliver genetic and epigenetic miRNA therapeutics. Finally, we discuss future directions, including theranostics, combinatory therapy, and personalized medicine.

Highlights

The obesity epidemic is a major public health crisis due to metabolic complications of obesity, such as insulin resistance and dyslipidemia, known as metabolic syndrome.

Nanotechnology methods of nanoemulsions, liposomes, and micelles offer advantages for molecular delivery, including targeting capacity, dissolution rate enhancement, and controlled release.

These advantages can help with addressing the obesity epidemic by patented food processing techniques that improve dietary quality through nutrient delivery as well as improved palatability of healthy foods.

The same nanotech delivery vehicles could also be used to deliver genetic and epigenetic therapeutics, such as anti-miRNAs blocking miRNAs implicated in obesity-induced insulin resistance.

Strategies to Fight Obesity

Obesity is an epidemic chronic disease affecting approximately one in three American adults and one in five American children [1]. Due to its related comorbidities (heart disease, diabetes, chronic liver disease, stroke, cancer, and Alzheimer's disease) it incurs medical costs (in the USA ~$340 billion annually or ~$3500 per person with obesity) [2] and lost workplace productivity due to factors like absenteeism (~$14 billion annually), disability, and premature mortality (~$30 billion annually) [3]. Nutritional quality is emphasized as a high priority goal to fight obesity [4]. Unfortunately, adherence to healthy eating has been low [5]. Innovative solutions to promote adherence to nonobesogenic dietary strategies are therefore needed.

There is also a need to identify pharmacological therapeutic approaches to treat or prevent obesity and **metabolic syndrome** (see Glossary). Another unexplored possibility lies in genetic therapeutics because there is a genetic component to these complex diseases [6]. A third and perhaps even more promising unexplored possibility is **epigenetics**, because population genetic changes alone cannot have caused the recent epidemic of metabolic syndrome [7]. Despite this potential, delivery of genetic and epigenetic therapies by traditional **viral vector methods** leaves them exposed to the body's defense mechanisms, which leads to diminished efficacy. There is a critical need for technology to encapsulate this material to protect it from degradation and uptake by the **reticuloendothelial system** in the circulation. These include physical methods that inject the genetic–epigenetic materials directly into the host cell (e.g., **electroporation**, **gene guns**), as well as chemical methods that surround the materials with protective molecules, such as the nanotechnology methods discussed below (Box 1). These methods have successfully delivered DNA or small noncoding RNAs for treatment of cancer [8], hepatitis C [9], and skin or stomach wounds [10]. However, there is very little evidence for treating metabolic disease, including obesity. In this review, we discuss progress toward the role of nanotechnology in this development and potential resolutions to the obesity epidemic.

Nanotechnology

Nanotechnology shows one of the most promising functional avenues for the development of advanced drug and gene delivery vehicles in fighting this epidemic [11–15]. The first such function is that nanosized materials can encapsulate therapeutic and diagnostic moieties, enhancing their physiological stability from the body's defense mechanisms. Compared with injected naked ther-

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Box 1. Nanotechnology and Chronic Diseases

Nanotechnology has the potential to enhance current medicines and methods for preventing, controlling, and mitigating diseases through drug delivery, sensors, and medical imaging because of its multifunctional aspects. Generally, biocompatible spherical nanomaterials act as carriers that can deliver the active agents located inside their hollow interiors, with sensors on their surface that detect a diseased area or cell, so that they release the active agents only at the targeted area for the therapeutic effect. In our opinion, this is one of the objectives when targeting fatty liver disease or fat tissues in obesity.

apeutic agents, **nanoparticle**-encapsulated ones have improved systemic half-life. Second, this encapsulation, along with specific targeting efficacy, enables therapeutic agents to accumulate into the local disease area, thus minimizing systemic toxicity. Otherwise these agents would be distributed into most body organs, requiring significantly higher dosing that increases systemic toxicity. Third, nanotechnology-based biosensors or imaging devices may be able to isolate diseased areas from normal body conditions because their targeting efficacy can significantly accumulate them in the diseased area, leading to sensitivity of disease detection. Thus, nanotechnology may have strong potential to control diseases. Compared with cancer therapy-related nanotechnology, much less research has addressed anti-obesity nanotechnology. However, scientific interest in the latter has increased dramatically over the past decade as evidenced by the number of related publications annually. Numerous investigations have shown that the most beneficial application of nanotechnology is controlling both tissue and cell distribution profiles of drugs by their entrapment in nanoparticles [16–20]. The rationale behind this approach is to increase therapeutic efficacy while reducing systemic side-effects. We speculate that this can be used to our advantage to treat deregulated metabolic tissues.

Despite several advantages (Box 2), nanotechnology also has some limitations. To be effective in the ways just described, nanoparticles must be stable as an individual particle during storage and postinjection. However, due to their large surface-area-to-volume ratio, nanoparticles become

Box 2. Advantages of Nanotechnology

The first advantage is increased dissolution rate. Most pharmaceutical entities are poorly water-soluble, which limits their bioavailability and ability to reach therapeutic levels in the body. Drug designers have overcome this issue by decreasing the particle to nanosize (10–1000 nm), increasing the surface area and surface curvature, thus reducing the width of the **diffusion boundary layers** and increasing the dissolution rate.

The second advantage of nanotechnology is improved solubility. For example, because glimepiride (an antidiabetic agent) has low aqueous solubility (e.g., 0.3–6 μg/m), it is typically co-administered with solubilizing agents that often cause severe side effects and drug fluctuations in the body [87–89]. However, encapsulating glimepiride in nanocapsules enhances its aqueous solubility by several orders of magnitude (e.g., ~2 mg/ml) without the toxic side effects of solubilizing agents and confers improved pharmacokinetic stability by protecting glimepiride from degrading [90,91].

The third advantage of nanotechnology is the protection of agents against the host's immune system by '**PEGylation**' [92]. This protection prolongs their time in circulation by reducing renal clearance in the body. Thus, the agent is better able to permeate and be retained in body tissue, improving the ability to provide the drug to the disease area and increase its therapeutic potency. However, PEGylation is not the only protection against host immunity in nanotechnology. Several studies are ongoing to prevent immunogenicity of nanoparticles, including hybridizing albumin with the nanoparticle [93], delivering cytokines or proteins that neutralize host immunogenicity [94], and manipulating agent geometry using the nanoparticle [95]. However, they were not approved by the FDA, which prevents their use in clinical trials.

A fourth advantage is safety because nanotechnology can mitigate the initial rise in drug concentration for controlled and sustained drug release and reduce dose variability by controlling nanoparticle composition, types of nanoparticles, and formulation methods.

Finally, the fifth advantage of nanotechnology is specific organ- and disease-targeting capacity. Targeted drug delivery by a nanoparticle provides significant local levels of therapeutic compounds with minimum systemic levels. Thus, only a low dose of the drug is required to achieve its therapeutic efficacy. This is one of the primary characteristics required to treat nonalcoholic fatty liver, which is one of the major dysregulations in obesity.

Glossary

Adipocytes: cells that are specialized primarily for the storage of fat.
Atrial natriuretic peptide: a peptide hormone that increases renal sodium excretion to reduce an expanded extracellular fluid volume.
Block copolymer: a chain of two or more polymers linked by covalent bonds.
cAMP: a common biological second messenger.
Co-solvent: a substance that makes two other substances more miscible when added to them.
Co-surfactant: a chemical that makes a surfactant more effective.
Diffusion boundary layers: the boundary layer of a particle characterized by a transverse concentration gradient of a given component that is contained in both the particle and its surrounding environment.
Dissolution rate: the release rate of a compound under a given set of conditions.
Drug loading capacity: the amount of a drug that a nanoparticle can carry.
Electroporation: the application of electric force to increase the permeability of the cell membrane, allowing chemicals, drugs, or DNA into the intracellular area.
Emulsion: droplets of a liquid into another liquid when the two liquids are not soluble or miscible.
Epigenetics: the interaction between genes and the environment that leads to changes in the regulation of gene expression.
Excipient: an inactive substance that acts as a vehicle for an active substance.
Gene gun: a system that coats DNA around a heavy metal to facilitate its injection into a cell.
Insulin resistance: dampening of the body's response to the hormone insulin, resulting in less storage and greater release of blood glucose. Can be induced and/or exacerbated by obesity.
Ionic complex: a chemical reaction whereby ions of opposite electrical charge come together in solution to form a distinct chemical entity.
Magnetoliposome: the combination of a liposome with a magnetic nanoparticle to create a multifunctional vesicle.
Metabolic syndrome: the clustering of the cardiovascular disease risk factors obesity, hypertension, insulin resistance, and dyslipidemia.

settled and caked, alter size, and form clusters (i.e., aggregate themselves) [21]. Aggregation during systemic injection lessens the nanoparticle's therapeutic advantage, while during storage limits shelf life, as nanoparticles settle and form cakes, rendering them ineffective. These stability issues require systemic toxic levels of **excipients** that synthesize or maintain the particles at the required size. There also remain some critical fundamental limitations of nanotechnology specific to drug delivery systems despite their significant progress [22]. These include the inability to: (i) precisely control shape, aspect ratios, size, and **polydispersity** of the nanocarriers; and (ii) integrate a variety of disease-specific triggered release mechanisms into the nanoparticle design. Overall, nanotechnology-driven drug and gene delivery systems have strong potential to control obesity effectively if researchers can overcome these limitations.

Specific Methods

Several current nanoparticle formulation methods are known to achieve the advantages described above. Therefore, given that nanotech delivery of genetic and epigenetic drugs for obesity is a new concept, traditional nanotechnology methods should be the first steps to apply toward obesity prevention/treatment (Box 3).

Nanotechnology Applications to Obesity

Pharmaceuticals

Nanotechnology is much more advanced in cancer prevention than obesity and diabetes due to several possible reasons: infancy of biomarker discovery, multiplicity of pathways, requirement of sophisticated control of drug release, and difficulty of targeting access. Nanotech vehicles can deliver cytotoxic agents to cancerous cells at lower doses and greater bioavailability than chemotherapy. They can furthermore deliver diagnostic antibodies to the surface of individual cancer cells using distinctive enhanced permeability and retention effect-mediated tumor access,

Box 3. Nanotechnology Methods

Nanotechnology methods fall into two categories: attrition and precipitation [96–100]. Attrition refers to breaking large particles into nanoparticles by physical means, such as a high-power air blow or ultrasound, while precipitation grows small particles from solutions until they reach the desired size. Besides its relative simplicity, precipitation is advantageous because it can achieve particle sizes <100 nm through the choice of materials. Better growth-rate control can be achieved using hydrophobic **polymers** or hydrophobic–hydrophilic copolymer compounds that do not crystallize easily.

Since control of growth rate is paramount for effective drug delivery nanoparticles, which must be highly stable at small sizes (<50 nm), it is best to use hydrophobic compounds or polymers that **self-assemble** and thus regulate the particle's growth rate by thermodynamics. To initiate this self-assembly, compounds or polymers are dissolved together with the agent they are intended to carry in organic solvent, then precipitated into water. The resulting molecule is called an **emulsion**. Besides their advantageous stability at small sizes, emulsions are easily and cheaply fabricated, and furthermore can be specifically engineered to deliver higher concentrations of pharmaceutical agents to desired locations. For these reasons, emulsions are ideal candidates for therapeutic agents against a specific disease such as obesity.

There are three specific types of emulsions: nanoemulsions (colloidal particles that are either an oil-in-water or water-in-oil dispersion, with emulsifying agents as **surfactants** to provide thermodynamic stability [101,102]); liposomes, which have a lipid bilayer [103,104] and can encapsulate either hydrophilic agents (e.g., RNA), hydrophobic agents, or both simultaneously; and micelles (composed of hydrophilic polar regions and hydrophobic inner regions).

Both nanoemulsions and liposomes use lipids originating from one of the cell membrane components, and both formulations tend to fuse with cells indiscriminately during systemic circulation [105,106]. This nonspecificity is sometimes not optimal for systemic application but can be overcome by attaching poly(ethylene glycol) (PEG) to their surface, which induces a 'stealth' property with minimal or no uptake by the reticuloendothelial system [107–109]. For example, a **block copolymer** of poly(Lactide-co-glycolide) (PLGA)-PEG tends to form a micelle but requires more chemical synthesis and purification [110,111]. Micelles generally are optimal for the encapsulation of hydrophobic agents, their stability is better than nanoemulsions and liposomes, and the formulation process is also simpler. Encapsulation of hydrophilic agents (e.g., DNA, RNA) within a micelle requires additional preformulation, of an **ionic complex** between RNA and a cationic polymer that must be accomplished before or during the formulation of the micelle [112,113].

Mitogen-activated protein kinase (MAPK): a family of serine/threonine protein kinases.
Nanographene oxide: a nanosized graphite particle, created by chemical exfoliation and separated into multiple size distributions using intense sonication and sucrose density gradient centrifugation.
Nanoparticle: a particle of dimensions less than 100 nanometers.
PEGylation: attachment of a polyethylene glycol polymer so that it coats a nanoparticle.
Pharmacodynamics: the study of the biochemical and physiologic effects of drugs.
Pharmacokinetics: the time-course of drug absorption, distribution, metabolism, and excretion.
Polydispersity: distribution of molecular mass within a polymer sample.
Polyethylene glycol (PEG): a polymer that consists of multiple ethylene glycol units.
Poly (lactic-co-glycolic acid) (PLGA): a polymer that consists of multiple glycolic acid and lactic acid units.
Polymer: a molecular structure comprised of similar units bound together.
Resveratrol: a natural phenol that has anti-inflammatory and antioxidant properties and is recognized to have therapeutic potential for several diseases, including cancer and diabetes.
Reticuloendothelial system: a part of the immune system that consists of the phagocytic cells located in reticular connective tissue.
Self-assemble: parts of a system assembling themselves into a larger unit.
Short-hairpin RNA (shRNA): an artificial RNA molecule with a tight hairpin turn that silences target gene expression by interfering with coding RNA.
Small inhibitory RNA (siRNA): a double-stranded RNA, 20–25 base pairs long, that interferes with the expression of target genes with complementary nucleotide sequences.
Surfactant: a chemical that reduces surface tension of another chemical when dissolved into it.
Viral vector methods: use of a virus as a vehicle to deliver genetic material to an organism.

allowing for greater diagnostic sensitivity and specificity than gross tumor imaging [23–25]. However, one of the biggest challenges in obesity is the specific delivery of the drugs, genetic, or epigenetic material to the targeted organ, including adipose tissues. Recent evidence shows some potential where nanoparticles were: (i) used to target the angiogenic vessels in adipose tissues [26], (ii) coated with the peptide (CKGGRAKDC) to target white adipose tissue [27], or (iii) coated with the peptide (KGGRAKD) to target the adipose vascular marker prohibitin [28]. A PubMed search for terms related to nanotechnology and cancer revealed 43 690 articles but, for obesity, <1% of this total.

Nonetheless, the advantages of liposomes, micelles, and nanoemulsions can be exploited to deliver drugs addressing obesity and its metabolic syndrome consequences [11–15]. For example, the fat absorption inhibitor orlistat blocks intestinal lipase but has very limited bioavailability since it is poorly water soluble. Nanoemulsions are hydrophilic but can encapsulate lipophilic agents, thus making them more water soluble, and have been successfully applied *in vivo* with orlistat [29]. Regarding metabolic syndrome consequences, toward treatment of type 2 diabetes, researchers have successfully delivered insulin orally (rather than the traditional subcutaneous pathway) by encapsulating it with liposomes. Since liposomes tend to fuse with any cell in the systemic circulation, the liposome–insulin complex fuses with cellular membranes and penetrates them more efficiently than insulin would by itself. This increases the biological action of insulin without the need for subcutaneous injection [11]. As a second example, toward treatment of hypertension, researchers have encapsulated the hydrophobic drugs candesartan cilexetil (an angiotensin II receptor blocker) and nimodipine (a calcium channel blocker) in micelles, which increased **drug loading capacity** and drug release, presumably due to increased aqueous solubility [12]. Additionally, the lipophilic antihypertensive drug olmesartan medoxomil (an angiotensin II receptor blocker) has been encapsulated in oil-in-water nanoemulsions, which increased its thermodynamic stability and **pharmacokinetic** activity [30]. In summary, the advantages of nanotechnology methods discussed in the previous section, including increased bioavailability, **dissolution rate**, control over drug release rate, and thermodynamic stability, can enhance pharmacotherapies for metabolic syndrome.

Nutraceuticals

Nanotechnology holds several potential critical applications in the medical field. However, another field which can benefit is nutraceuticals. A nutraceutical is defined as a food or part of it that provides the body with medical or health benefits, including the prevention and treatment of a disease. This is an important area to explore in obesity prevention, as nutrition plays a prominent role in this epidemic. The ability of nanoemulsions and micelles to increase the dissolution rate and aqueous solubility of bioactive molecules is therefore advantageous to delivery of not only drugs but also nutraceuticals, to increase diet quality. Many nutraceuticals, such as vitamins, minerals, antioxidants, fats, and proteins, are currently in common edible ingredients, such as cooking oil. Some of them are frequently poorly absorbed during digestion, however, due to being more fat soluble than water soluble, thus hindering dietary benefit for the consumer [31]. These nutraceuticals with high fat solubility cannot be digested until fat has been digested, and during this delay are subject to degradation. Thus, nanoemulsions and micelles, which successfully increase the water solubility of nutraceuticals, increase the bioavailability of those nutraceuticals and absorbed nutritional quality.

Recent reviews [32], including two comprehensive lists of existing patents [33,34], have listed foods that address this purpose of improving nutrient delivery through nanotechnology-enhanced water solubility. As discussed earlier in this review, the choice of encapsulating agent determines the type of nanoparticle formed. 'Dried formulations' [35] use solubilizing agents such as polyoxyethanyl tocopheryl sebecate (PTS^{TM}) to form micelles around fat-soluble nutrients like coenzyme Q10, omega fatty acids, vitamin B6, and **resveratrol**, thus increasing their water solubility and improving delivery. Resveratrol delivery can be further improved by solid lipid

nanoparticles and nanostructured lipid carriers that increase its intestinal permeability [36]. Another patent 'Nano-sized self-assembled liquid dilutable vehicles' [37] describes a multiphase nanoemulsion, including both aqueous and oil phases, which the inventors claim increases water solubility 7–20 times above single-phase nanoemulsions. These techniques have applied the nanoencapsulation self-assembly procedures, due to their advantages of lower cost and better specificity of sizing and location of particle delivery compared with the attrition or precipitation techniques. Overall, the increased bioavailability of healthy nutrients using micelles and nanoemulsions addresses the problem of metabolic syndrome by improving dietary quality and thus reducing the caloric intake required to obtain adequate nutrition.

Furthermore, these nanoscale food processing techniques address the metabolic syndrome not only nutritionally but also behaviorally by improving the palatability of healthy foods through their flavor and texture. The Slim Shake Chocolate by Nanoceuticals™ (http://www.nanotechproject.org/cpi/products/nanoceuticalstm-slim-shake-chocolate/) adds pure cocoa to its nanoencapsulations to enhance flavor without having to add pure sugar. Similarly, Nestle and Unilever used nanoemulsions to create ice cream with 1% instead of 16% fat content without compromising taste [38]. In summary, nanoencapsulation self-assembly techniques have the potential to increase not only the benefits of eating nutrient-rich foods but also the likelihood that people will prefer them to less nutritious and otherwise more palatable foods.

Another less explored nanotech approach to preventive nutrition for metabolic syndrome that may warrant further consideration is dietary supplementation with silver (Ag) nanoparticles. These are widely used in food storage, environmental sterilization, and animal husbandry due to antimicrobial properties. However, in the latter case, a recent study reported they also enhanced muscle growth of the animals (poultry), along with decreased plasma cholesterol, triglyceride, and glucose but no change in fat growth [39]. These results were attributed to the Ag nanoparticles' anti-inflammatory actions [40], digestion stimulation [41], and promotion of growth factor transcription (*IGF1*, *Glut1*, *Glut3*). Similarly favorable metabolic changes from Ag nanoparticles were recently observed *in vivo* in a mouse model [42]. At the same time, other studies found dietary supplementation with Ag nanoparticles did not influence livestock growth [43–46]. In our opinion, more research is warranted and it is possible that Ag nanoparticle size, dose, exposure time, and/or preparation method may relate to these discrepancies.

In spite of the potential advantages, nanoscale food processing also poses potential health hazards that could cause adverse responses regarding metabolic syndrome phenotypes. The primary health concern we predict is the lack of knowledge about how the human body digests and absorbs these ingested nanoparticles. Molecular properties, such as concentration, particle size distribution, and electrical charge, are different on a nanoscale compared with traditional microscale nutraceuticals, which can affect digestion and absorption of the nanosized materials. This difference is concerning because some nanosized materials contain digestible obesogenic molecules (e.g., lipids in liposomes, carbohydrates in lipopolysaccharides). However, lower-energy nanoemulsions can be formed if **co-solvents** (e.g., propylene glycol) or **co-surfactants** (e.g., short- and medium-chain alcohols) are added [32].

Another concern is that nanoemulsions also contain nondigestible inorganic nanoparticles, including minerals and metals, which may influence biological pathways that contribute to metabolic syndrome such as inflammation. As recently reviewed by Siddiqi *et al*. [47], silver nanoparticles generate free radicals and reactive oxygen species that inhibit microbial growth. They can also block dietary supplements from otherwise scavenging these radicals [48]. It is unknown whether these free radicals could adversely affect human cells by reducing ATP content, increasing reactive oxygen species production, and/or damaging mitochondria and DNA. Such

disruption of cellular signalling could exacerbate metabolic syndrome, if these free radicals and reactive oxygen species increased the inflammation that contributes to obesity-induced **insulin resistance**. These concerns are mitigated by animal models suggesting that silver nanoparticles undergo fecal elimination before accumulating in toxic doses [49].

Despite the growing number of patents supporting the benchtop efficacy of nanoscale food processing and the potential benefits of this technology as lifestyle therapy for metabolic syndrome, however, no human clinical trials exist to verify whether potential benefits outweigh risks. Nonetheless, these safety issues must be weighed thoughtfully when evaluating the risk-to-benefit ratio of using nanocarriers to deliver obesity therapeutics. Unlike malignant cancers more commonly treated by nanotechnology, obesity is a nonmalignant condition and so the benefits of treating it do not necessarily outweigh the safety risks.

At the same time, it is important to recognize that nanocarriers can also improve the safety of both drugs and nutraceuticals by lowering their systemic toxicity through two mechanisms: (i) increased targeting efficacy, and (ii) decreased drug carrier toxicity. Targeting efficacy of nanoencapsulated therapeutics can be enhanced by the choice of disease-specific ligands coated on the nanoparticle. Additionally, the toxicity of the drug carrier is totally dependent on the nanoparticle toxicity. Currently, there are two FDA approved biomaterials, **poly (lactic-co-glycolic acid) (PLGA)** and **polyethylene glycol (PEG)**. Nanoparticles composed of PLGA or PEG or a combination of both could minimize the systemic toxicity.

Besides safety considerations, we must consider whether nanocarriers can feasibly deliver drugs and nutraceuticals to have positive impact on these phenotypes. In particular, obesity and metabolic syndrome are chronic diseases that may require continuous delivery of therapeutics, in contrast to acute conditions such as cancer that can resolve with several courses of acute therapy. To determine if nanocarriers can address this challenge, their maximum tolerated dose must be determined because it guides the doses and even frequency of new therapeutics. Compared with naked therapeutics, the maximum tolerated dose of nanoencapsulated therapeutics is generally higher because the nanoparticle can protect the encapsulated therapeutics during systemic injection. It can consequently delay the release of therapeutics from nanoparticles, in turn decreasing the dosing frequency required to achieve continuous delivery. Thus, nanocarrier applications could achieve feasible effects in obesity and metabolic diseases.

Genetic Therapeutics

Beyond these applications of nanotechnology to deliver drugs and food, nanotechnology also bears the potential to deliver genetic material [14]. Nanotech methods can be used to encapsulate DNA so that it can be therapeutically delivered, without exposure to the host's immune system as we see with traditional viral gene delivery. Nanotech delivery also averts the risk of recombination between the therapeutic gene and the vector, as can occur with viral gene delivery. It also confers other advantages over traditional viral gene delivery, including unlimited length of the therapeutic DNA sequence, as well as increased target specificity and high delivery efficacy [14], just as we see with nanotech-delivered drugs and nutraceuticals.

As with drug delivery, DNA delivery by nanotech has been studied more extensively as therapy for cancer [14] than obesity or metabolic syndrome. Nonetheless, the approach bears future potential for obesity. Recently, Park *et al*. used nanoparticles to deliver an anti-obesity gene construct (pDNA) for anti-obesity in diet-induced obese mice [50].

By working at the genetic level, it is possible to impact multiple pathways, a concept known as synthetic biology, meaning the manipulation of an entire biological system as opposed to a single

biological pathway. This approach could be particularly beneficial in the context of metabolic syndrome, which is characterized by multiple abnormalities including obesity, hypertension, insulin resistance, and dyslipidemia that do not necessarily arise from a single underlying causal etiology [51]. This clustering of multiple abnormalities in patients with metabolic syndrome thwarts many common pharmaceutical therapies that only target one of them, for example, antihypertensive drugs that lower blood pressure but do not impact lipid profile or insulin sensitivity.

However, recent experiments in mice support the possibility of gene therapy solutions to address multiple metabolic abnormalities concurrently. Ye *et al*. [52] demonstrated that by pharmacologically inducing **cAMP**-dependent signalling, they increased genetic expression of GLP-1-FcmIgG-Leptin, a polypeptide containing glucagon-like peptide 1 (GLP-1) and leptin. GLP-1 stimulates insulin secretion and leptin signals satiety, thus impacting insulin resistance and obesity, respectively and simultaneously. Thus, manipulation of GLP-1-FcmIgG-Leptin expression at the genetic level represents a potential future strategy to treat the metabolic syndrome at the biological system level, rather than treating a single component of the metabolic syndrome (e.g., insulin resistance) at the single biological pathway level. If future studies could use nanotech methods to deliver a grafted DNA sequence that increases GLP-1-FcmIgG-Leptin expression, this work may lead to the evolution of future genetic nanotech pharmacotherapies for metabolic syndrome.

Even such target genes that influence multiple metabolic syndrome components, however, are limited by their open-loop mechanism, meaning that no counter-regulatory mechanism exists to preserve homeostasis. Synthetic biology can address this limitation as well, however, by creating a closed-loop system in which autoregulatory feedback controls metabolic abnormalities. For instance, Ye *et al*. [53] treated mice with obesity-induced insulin resistance by implanting a synthetic sensor-effector device capable of sensing heightened insulin levels and accordingly releasing the insulin-sensitizing hormone adiponectin to restore insulin sensitivity. The device worked by: (i) adjusting **mitogen-activated protein kinase (MAPK)** signaling such that heightened insulin levels caused MAPK to activate a transcription factor [tetracycline repressor fused to ELK-1 derived transactivation domain (TetR-ELK1)]; and then (ii) creating synthetic promoters such that TetR-ELK1 modulated gene expression to stimulate appropriate adiponectin release, thus restoring insulin sensitivity, completing the closed-loop mechanism. Such a closed-loop glycemic regulatory system has also been achieved for human cells *in vitro*, by coupling glycolysis-mediated calcium entry to GLP-1 transcription, such that heightened glucose levels stimulated increased glucose uptake (i.e., 'mimetic β-cells') [54].

Closed-loop genetic therapies have also been studied for regulation of satiety, lipid levels, and blood pressure in the presence of overfeeding and/or hyperlipidemia. Rossger *et al*. [55] treated mice via implanting cells containing the lipid receptor peroxisome proliferator-activated receptor-α (PPAR-α) grafted to bacterial DNA-binding repressor TtgR that stimulates expression of the satiety hormone pramlintide. This approach led to these cells functioning as lipid-sensing receptors causing the mice to experience increased satiety in the presence of hyperlipidemia, yielding overall reductions in caloric intake and lipid levels. In a separate study, Rossger *et al*. [56] genetically rewired the dopaminergic system such that the presence of food stimulus triggered the release of **atrial natriuretic peptide**, thus regulating blood pressure in hypertensive mice. In summary, researchers have developed genetic therapeutics that each address multiple metabolic syndrome components, including obesity [52,55], insulin resistance [52,53], dyslipidemia, and hypertension [55]. If the synthetic genes for these methods could be delivered using nanotech methods, the advantages of nanotech could be harnessed, including increased target specificity and high delivery efficacy, and furthermore eliminate the need for viral DNA vectors that can recombine with the therapeutic gene. For example, an oligopeptide complex for targeted nonviral gene delivery to

adipocytes showed regression of obesity and obesity-induced insulin resistance [57]. A polyionic complex containing a gene system engineered to promote energy expenditure through the conversion of white adipocytes into beige/brite adipocytes (i.e., browning) demonstrated similar results [58].

Traditional viral gene delivery also risks the possibility of recombination between the therapeutic gene and the viral vector, which is not a possibility with nanotech delivery. Nanotech gene delivery also confers other advantages over traditional viral gene delivery, including delivery of unlimited length of DNA sequence, as well as increased target specificity and high delivery efficacy [14], just as we see with nanotech-delivered drugs and nutraceuticals.

Epigenetic and miRNA Therapeutics

Epigenetics, the interaction between genes and the environment, is perhaps an even more promising unexplored possibility for obesity therapeutics than genetics because epigenetic changes happen before genetic changes and they are reversible [59,60]. Our previous review covers epigenetic modifying drugs that address obesity and metabolic syndrome by targeting histone acetylation, DNA methylation, and sirtuin activation [59]. In the present review we turn this discussion to the potential for small noncoding RNAs (another subset of the epigenetics concept) to be added to this list of therapeutic targets, since nanotech vehicles may turn out to be especially vital to this process.

Beyond the potential for delivery of DNA, nanoemulsions, liposomes, and micelles have the potential to deliver RNA (also a hydrophilic agent). This is an important potential application of these nanotechnology vehicles because small noncoding RNAs bear strong therapeutic potential for metabolic syndrome, but delivery remains a major challenge. This section will overview the therapeutic potential of small noncoding RNAs, challenges that preclude using them as therapies for metabolic syndrome in humans, and potential nanotechnology solutions to these challenges.

The type of small noncoding RNAs most explored from a therapeutic perspective are miRNAs: short (~22 nucleotides), single-stranded noncoding gene products which regulate gene expression. miRNAs contribute to many essential biological processes like development, energy and lipid metabolism, insulin secretion, adipocyte differentiation, and arterial blood pressure regulation [61,62]. They are aberrantly expressed in pathophysiological conditions like obesity, diabetes, and heart disease and can act as biomarkers for the diseased state [61,62]. Thousands of unique miRNAs exist, and each miRNA can target multiple genes, and each gene can influence multiple metabolic pathways, suggesting the strong prognostic and therapeutic importance of miRNAs for human diseases.

Chakraborty *et al*. [63] conducted a systematic review and identified over 1000 patents related to therapeutic use of miRNA. These technology developers are following the standard drug development process: discovery research, **pharmacokinetics**/**pharmacodynamics** testing, and large animal efficacy studies, before moving to human clinical trials. The most popular molecular approach has been the construction of antisense oligonucleotides (anti-miRs) that bind specific miRNA transcripts, thus preventing those miRNA transcripts from regulating gene expression and achieving therapeutic impact on biological pathways involving those genes. Since miRNAs are short (21 base pairs) and highly conserved across species, anti-miRs have the potential to achieve much higher specificity than drugs that target DNA, the target sequence of which is likely to be longer and more variable between individuals.

Among all the miRNA patents Chakraborty *et al*. [63] identified, the disease most widely targeted is cancer. The single furthest developed therapeutic anti-miR to date, however, is anti-miR-122,

which targets hepatitis C virus (HCV) and has reached Phase II human clinical trials. Patients with HCV receiving anti-miR-122 injections for 1 month achieved prolonged dose-dependent reductions in both HCV RNA and miR-122 over 4 months [64]. The investigators also analyzed a panel of 179 other miRNA transcripts found in plasma for possible off-target pleiotropic side effects [65]. Although two nontargeted transcripts (miR-210 and miR-532-5p) changed acutely following the month of anti-miR-122 injections, the changes were of much lower magnitude and much less prolonged in duration than the changes in the target transcript miR-122 [65]. These findings are encouraging regarding potential use of miRNA for obesity, however caution is advised regarding side effects on other miRNAs.

The question is whether science can apply this therapeutic potential of anti-miRs exemplified by anti-miR-122 toward treatment for obesity and metabolic syndrome in humans, at which point these anti-miRs could be delivered via liposomes. There are several miRNAs that are: (i) dysregulated during obesity *in vivo* within adipocytes or other tissues (hepatocytes, pancreatic islets, hypothalamus, or circulating fluids); (ii) associated with insulin resistance within adipocytes, skeletal muscle, and/or liver; and/or (iii) associated with other aspects of obesity progression potentially influencing insulin resistance within adipocytes (adipogenesis, lipid metabolism, inflammation) or liver (lipid metabolism, steatosis).

These miRNAs represent promising therapeutic targets for new anti-miR drug technology that could address metabolic syndrome, but the standard concerns of drug discovery must be addressed, including both pharmacokinetics/pharmacodynamics issues (tissue permeability, stability, target miRNA binding affinity) and off-target toxicity issues. A single miRNA can regulate genes in multiple biological pathways, so even an anti-miR with high binding specificity to its target miRNA may induce unwanted pleiotropic side effects. Another concern is whether anti-miRs can achieve enough tissue permeability and *in vivo* stability to functionally block their target miRNA as intended.

Traditional strategies to alter the pharmacokinetics and pharmacodynamics of anti-MiRs include chemical modification of the sugar–phosphate backbone or the nucleotides themselves [66]. However, these concerns may be better addressed by nanotechnology. Encapsulation of an miRNA or anti-miR into a nanoparticle (e.g., liposome) can protect them from instability and their off-target tissues [67]. For example, **nanographene oxides** have been used to deliver let-7g mimics [68], as well as anti-miR-21 [69] to cancer cells.

Among all anti-miRs manufactured to date that have achieved enough stability *in vivo* to impact health outcomes among large animal models, the one that is pertinent to metabolic syndrome is 2′-fluoro/methoxyethyl modified anti-miR-33 (manufactured by Regulus Therapeutics, La Jolla, CA). Metabolic syndrome associates with lower plasma high-density lipoprotein cholesterol (HDL-C) and impaired reverse cholesterol efflux, leading to atherosclerotic plaque build-up. Studies in mice, as well as human cells *in vitro*, found that miR-33 regulates this process by targeting genes involved in HDL-C biogenesis and/or cholesterol efflux (*ABCA1*, *ABCG1*) [70–74]. Whereas, administration of anti-miR-33 increases HDL-C and cholesterol efflux [70–72], while reducing atherosclerotic plaque [75,76].

Therefore, Rayner *et al*. [77] extended this testing to primates, comparing changes in cholesterol profile between six African green monkeys receiving anti-miR-33 versus six receiving a placebo mismatched anti-miR over 12 weeks. Like the mice, monkeys receiving anti-miR-33 increased hepatic ABCA1 as well as plasma HDL-C, indicating reduced susceptibility to metabolic syndrome [78]. However, the investigators expected more pleiotropic effects of the drug among monkeys than mice because, while both species express miR-33a, only monkeys express the

sibling transcript miR-33b. MiR-33b originates from the same seed sequence as miR-33a but differs by two nucleotides. Therefore, the investigators also examined whether anti-miR-33 influenced target genes of miR-33b, including those related to fatty acid oxidation (*CROT*, *CPT1A*, *HADHB*, and *PRKAA1*) and synthesis (*SREBF1*, *FASN*, *ACLY*, and *ACACA*). Notably, the former increased expression whereas the latter decreased expression, resulting in lower plasma very low-density lipoprotein (VLDL)-associated triglycerides. In summary, the benefits of anti-MiR-33 for metabolic syndrome risk were enhanced even more among large versus small animals. These benefits could be reaped to a greater degree using delivery of miR-33b via liposomes to increase stability and target gene specificity in humans.

At the same time, caution is warranted because anit-miR-33 and/or genetic ablation of miR-33 in mice has led to detrimental effects over long-term experiments (~12 weeks) related to metabolic syndrome, including hypertriglyceridemia, hepatic steatosis, body weight gain, and insulin resistance [79–83]. Thus, efficacy trials over a longer portion of lifespan in non-human primates are required before moving to human clinical trials, as is improved target gene specificity that could be achieved using liposome delivery vehicles (Figure 1).

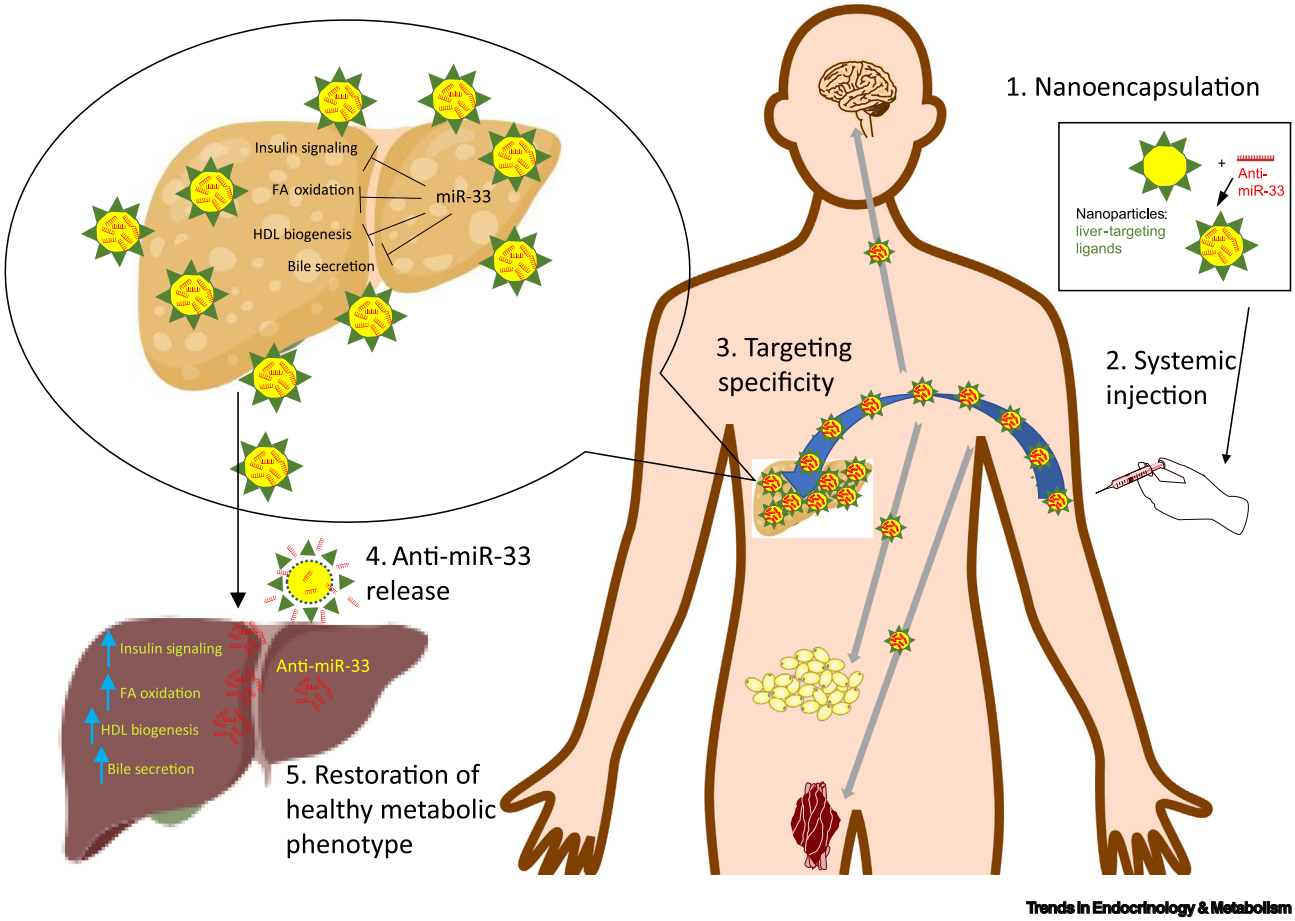

Figure 1. Proposed Steps of Delivering Nanoencapsulated Anti-miR-33 to Target the Liver. Nanoencapsulated anti-miR-33 could rescue fatty liver when delivered to the body. Increased miR-33 inhibits expression of genes in the liver that would otherwise stimulate insulin signaling, FA oxidation, HDL biogenesis, and bile secretion. Anti-miR-33 reverses these adverse metabolic effects of miR-33 and rescues fatty liver and its downstream effect. Due to the liver-specific coating, a significant portion would go to the liver and very little to other organs. Abbreviations: FA, Fatty acid; HDL, high-density lipoprotein; WAT, white adipose tissue.

A search of clinicaltrials.gov for 'microRNA AND (obesity OR dyslipidemia OR hypertension OR diabetes)' revealed 112 trials but none pertained to human trials of miRNA-targeting drugs. All pertained to miRNAs as disease biomarkers or biomarkers assessed for change in response to a standard clinical intervention for metabolic syndrome, either pharmacological (i.e., oral antihyperglycemic for diabetes) or behavioral lifestyle (i.e., diet or exercise). Progression toward FDA-approved nanotechnology for metabolic syndrome utilizing anti-miRs appears slow, likely due to not only the safety concerns but also the stability challenges described above. Thus, the new nanotech approach of encapsulation with liposomes to enhance stability holds promise to aid this process. As proof of this concept, a mimic of the oncogene inhibitor miR-34 has been successfully encapsulated in liposomes and injected into human cancer patients (MRX34, Regulus Therapeutics) [84], although the Phase I clinical trial of this agent was terminated early due to immune-related serious adverse events (https://clinicaltrials.gov/ct2/show/NCT01829971).

It is also noteworthy that beyond miRNAs, there are other small noncoding RNAs, including **short-hairpin RNAs (shRNAs)** and **small inhibitory RNAs (siRNAs)** that are also hydrophilic and can be carried by liposomes [85]. Supporting the possibility that these other small noncoding RNAs could be therapeutic for obesity, Won *et al*. [57] designed an shRNA that successfully silenced the fatty-acid-binding 4 gene (*shFABP4*) that otherwise facilitates the storage of lipid droplets in white adipocytes contributing to fat accumulation, which led to weight loss in obese mice. However, this shRNA has not been tested in larger mammals so, compared with miRNA therapies for obesity, the challenges in translation that will arise and the specific role that nanotechnology could play is less well known.

Concluding Remarks and Future Perspectives

Nanotechnology has shown promising efficacy in animal models to achieve more effective *in vitro* delivery of drugs addressing metabolic syndrome complications of obesity, including type 2 diabetes and hypertension. It can achieve nutraceutical delivery in humans to increase diet quality and the techniques could be harnessed to deliver genetic and epigenetic therapeutics for obesity and metabolic syndrome.

In summary, we suggest several future directions (Figure 2, Key Figure). The first direction is 'theranostics' that combine therapy and diagnosis within a nanoparticle. Thus, the diagnostic and therapeutic agents are encapsulated and delivered to the disease area. This leads to guided delivery that can identify the disease targeting area and also can track the progress of therapy. For example, **magnetoliposomes** loaded with polyunsaturated fatty acids have been demonstrated to both detect and attenuate inflammatory biomarkers in mice [86]. Along similar lines, it may be possible, for example, to encapsulate anti-miR-33, which can treat dyslipidemia, into a liposome also containing a detection agent for dyslipidemia.

Next, we suggest combinatory therapy: the delivery of both drugs and genes in the same nanotech delivery vehicle. This combinatory therapy could result in a synergistic effect that will yield benefits including enhanced therapeutic effects, diminished dose, and reduced side toxicity. For example, a single liposome could be used to encapsulate both insulin and synthetic DNA altering PPAR-γ expression to increase insulin sensitivity.

The final suggested direction is personalized medicine. Researchers and clinicians could tailor the targeted delivery of genetic and epigenetic therapeutics by nanotech vehicles to the genomic and epigenomic profile of the individual. For instance, anti-miR-33 may be more effective for individuals with higher levels of miR-33 and/or polymorphisms of its target gene *ABCA1*

Outstanding Questions

Can the encapsulation of anti-miRNA into liposomes or another nanotech delivery vehicle help achieve the pharmacokinetic stability and target gene specificity required to effectively treat obesity and its complications in humans, without detrimental side effects?

Will other such potential therapeutic agents, including shRNAs and siRNAs, that have shown efficacy in mice encounter similar challenges to anti-miRs when applied to larger mammals, such that nanotechnology delivery could aid in their translation to humans as well?

Can a precision medicine approach be applied to nanotech-delivered epigenetic therapies for obesity, to account for polymorphisms in their target genes?

Can nanotech delivery vehicles for obesity therapeutics be expanded to 'theranostics' and combinatory therapies?

Considering both the beneficial and adverse effects of nanotech-delivered nutraceuticals observed in animal models, will human clinical trials of their efficacy to treat obesity yield beneficial or adverse results?

Key Figure

Nanotechnology Approach to Obesity

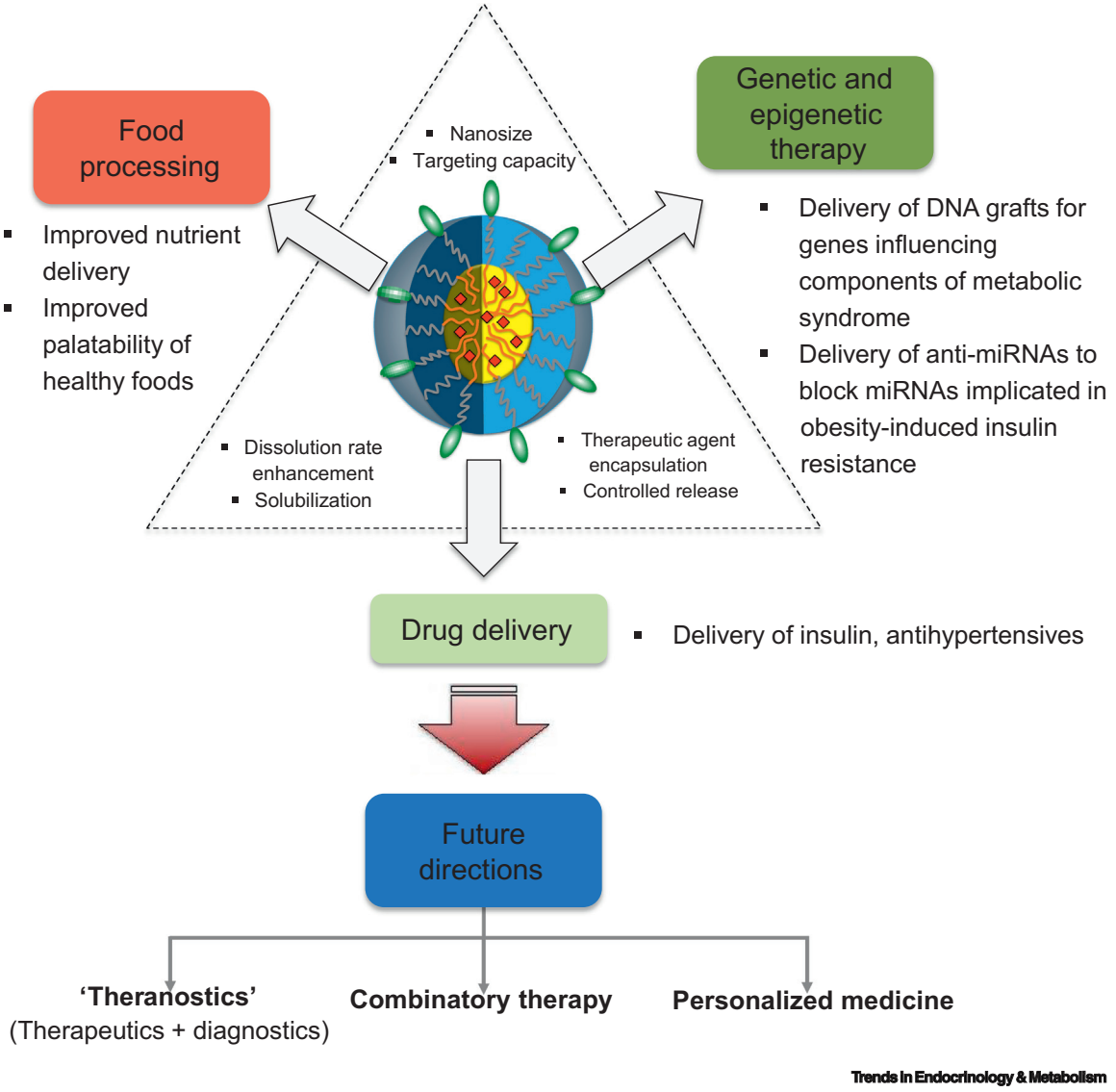

Figure 2. Schematic diagram for control of obesity by the unique properties of nanoparticles, such as targeting capacity, dissolution rate enhancement, and efficient encapsulation of various types of agents. Due to these properties, the nanoparticle can be applicable for food processing, genetic and epigenetic therapeutics, and drug delivery. Future applications include theranostics, combinatory therapy, and personalized medicine.

that increase binding with miR-33, whereas individuals with lower levels of miR-33 and/or not carrying those polymorphisms may benefit from different therapies. As science gains further knowledge of genomic variation and function pertinent to metabolic syndrome, more such possibilities will emerge.

In conclusion, we propose that the novel idea of nanotherapeutics in obesity could benefit a broad spectrum of research in the near future (see Outstanding Questions) aiming to prevent obesity and its comorbidities.

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