INTABIOTECH

HYDROXYTYROSOL FROM OLIVE EXTRACTS AND HEALTHY AGEING

From antioxidant biomarker modulation to the Hydroxytyrosol–DOPAC metabolic axis

Critical scientific review of human evidence, pharmacokinetics, formulation, analytical control and EU regulatory implications

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Abstract

Hydroxytyrosol (HT; 3,4-dihydroxyphenylethanol, DOPET) is a characteristic olive phenol whose human biology is more complex than the conventional label ‘antioxidant’ suggests. A 2025 randomized, double-blind, placebo-controlled trial in adults aged 40–70 years with overweight and prediabetes reported that 15 mg/day for 16 weeks favourably modified oxidized LDL, protein carbonyls, 8-hydroxy-2′-deoxyguanosine and selected antioxidant/inflammatory variables, while conventional glycaemic indices, anthropometry and the standard lipid profile were not materially improved. A 2026 untargeted metabolomic analysis of the same cohort identified changes involving arachidonic-acid metabolism, purine catabolism and membrane-lipid classes. Together, these findings strengthen the biological plausibility of HT as a modulator of processes associated with cardiometabolic ageing, but they do not demonstrate prevention of diabetes, cardiovascular events, neurodegenerative disease or ageing itself. [1–3]

A central translational issue is pharmacokinetics. Free HT appears rapidly in plasma and is extensively transformed by first-pass and systemic metabolism. Human studies repeatedly identify sulphated and glucuronidated HT, homovanillic acid and 3,4-dihydroxyphenylacetic acid (DOPAC) among the major products of exposure. After olive ingestion, plasma DOPAC can rise far more than free HT, supporting substantial conversion through the sequence HT → 3,4-dihydroxyphenylacetaldehyde (DOPAL) → DOPAC. This makes the ‘HT–DOPAC axis’ a scientifically useful model: oral HT should be understood as the entry point to a dynamic metabolite network rather than as a single circulating molecule. [4–7]

The regulatory conclusion is equally important. In the European Union, the authorised claim concerns olive-oil polyphenols and protection of blood lipids from oxidative stress, under defined conditions requiring at least 5 mg of hydroxytyrosol and derivatives per 20 g olive oil. This is not a general authorisation for isolated HT supplements. Moreover, in 2025 EFSA concluded that a cause-and-effect relationship had not been established between phenolic compounds naturally present in olive oil and lowering LDL cholesterol or systolic blood pressure for a proposed coronary-heart-disease risk-reduction claim. [8,9]

Bottom line
The current evidence justifies serious clinical and product-development interest in hydroxytyrosol, particularly in metabolically vulnerable populations. It does not justify wording that HT has been proven to prevent age-related disease. The strongest near-term scientific opportunity is to integrate standardized HT exposure with metabolite-resolved pharmacokinetics (including DOPAC), validated oxidative/vascular biomarkers and longer clinical trials.

Key Messages for R&D, Regulatory and Product Development

- The 2025 prediabetes trial is clinically relevant because it studied an at-risk population and used a 16-week placebo-controlled design, but its benefits were principally biomarker-level rather than improvements in glucose control or body weight.
- DOPAC is not a peripheral analytical curiosity: it is a major human metabolite of HT exposure and may materially contribute to the biological phenotype attributed to olive phenols.
- The pharmacological unit of interest should increasingly be considered an exposure profile (HT + conjugates + DOPAC + methylated metabolites), not merely milligrams of parent HT on the label.
- Evidence generated with olive oil, olive fruit/leaf extracts, purified HT and multi-ingredient formulas cannot be treated as interchangeable.
- For industrial development, identity, chemical form, matrix, dose, oxidative stability, DOPAC content, secoiridoid profile and batch-to-batch metabolite potential are all relevant quality variables.
- For EU commercial communication, the olive-oil-polyphenol health claim is matrix- and condition-specific; disease-risk or glucose-control claims for stand-alone HT are not established by the current evidence.

Contents

1. Scope and Evidence Framework

This review evaluates whether recent human evidence materially changes the scientific case for hydroxytyrosol as a nutritional intervention relevant to age-associated chronic disease. It is a targeted narrative review rather than a formal PRISMA systematic review. Priority is given to controlled human trials, human pharmacokinetic studies, EFSA opinions and current EU legislation; mechanistic and preclinical literature is used to interpret, not to replace, clinical evidence.

The phrase ‘may help reduce risk of age-related disease’ requires careful unpacking. Risk can be influenced through validated causal risk factors, intermediate biomarkers or broader biological processes, but these are not equivalent evidentiary categories. A reduction in oxidized LDL or 8-OHdG can support a mechanistic hypothesis without proving fewer myocardial infarctions, less diabetes or slower ageing. Throughout this document, disease-prevention language is therefore reserved for hypotheses unless a clinical endpoint or accepted risk-factor pathway has been demonstrated.

Corporate context
ND Pharma & Biotech Co. reports historical technical experience in the manufacture and use of hydroxytyrosol and DOPAC (3,4-dihydroxyphenylacetic acid). That experience is relevant to process development, analytical control and formulation strategy, but is not presented here as evidence of clinical efficacy.

2. Chemistry, Origin and Nomenclature

Hydroxytyrosol is a small catecholic phenylethanol (C8H10O3; molecular mass approximately 154.16 Da). Common synonyms include 3,4-dihydroxyphenylethanol and DOPET. Its adjacent 3,4-dihydroxyl groups confer a redox-active catechol motif capable of electron/hydrogen donation, metal interaction and oxidation to quinone-type species. This chemistry underlies both its antioxidant potential and its chemical instability under unfavourable processing or storage conditions.

In olives and virgin olive oil, HT exists both as free phenol and as part of more complex secoiridoid-derived structures. Oleuropein and related compounds can yield HT during fruit ripening, processing, storage and digestion. Consequently, ‘hydroxytyrosol exposure’ after olive consumption can derive from several precursor forms rather than only from analytically free HT in the starting material. [10–12]

DOPAC is 3,4-dihydroxyphenylacetic acid, another catechol carrying a carboxylic-acid side chain. It is widely recognized as a dopamine metabolite, but it is also produced from exogenous HT. This overlap between dietary phenol metabolism and endogenous catecholamine metabolism is one reason why metabolite-resolved analytical design is essential.

3. Prediabetes as a Model of Age-Related Cardiometabolic Risk

Prediabetes is not a homogeneous disease entity; it is a risk state defined by glycaemic criteria and frequently accompanied by visceral adiposity, dyslipidaemia, endothelial dysfunction, oxidative stress and low-grade inflammation. These features overlap with several biological processes that become more prevalent with ageing. Studying HT in this population is therefore more informative for prevention research than studying only young metabolically healthy volunteers, provided that the interpretation remains anchored to measured outcomes.

Oxidative modification of LDL, protein carbonyl formation and oxidative DNA damage are not interchangeable biomarkers. Each reflects a different biochemical compartment and each has methodological limitations. A coherent movement across several markers is more persuasive than a change in one isolated assay, but none of them independently establishes reduced disease incidence.

4. Human Pharmacokinetics: the Parent Compound Is Only Part of the Story

Human pharmacokinetic studies consistently show rapid appearance and rapid disappearance of free HT. In a 2010 study using purified HT in aqueous solution (2.5 mg/kg body weight), plasma HT peaked within minutes and was undetectable after two hours; urinary recovery included free and conjugated HT-related products, including DOPAC. The study also found transient association of HT with isolated LDL fractions, illustrating that distribution into lipoprotein compartments can occur even when free plasma residence is brief. [4]

A randomized crossover study using HT-enriched biscuits (5.25 mg HT) found plasma metabolites peaking at approximately 0.5–1 hour. HT-sulfate and DOPAC-sulfate were prominent metabolites, followed by DOPAC and homovanillic acid, with additional glucuronides detected. This is a key observation for translational science: the molecular species reaching tissues after oral intake are not limited to the parent compound. [5]

More recent supplement pharmacokinetic work likewise identified homovanillic acid, HT-3-O-sulfate and DOPAC among the principal metabolites in plasma and urine after olive-derived aqueous supplements. Exposure increased with dose, but substantial interindividual variability remained. [6]

Interpretive consequence
A formulation that delivers the same nominal milligrams of HT may generate a different biological exposure if its matrix, release kinetics, precursor profile, gut handling or first-pass metabolism alter the proportions of HT, DOPAC, sulfates, glucuronides and methylated metabolites.

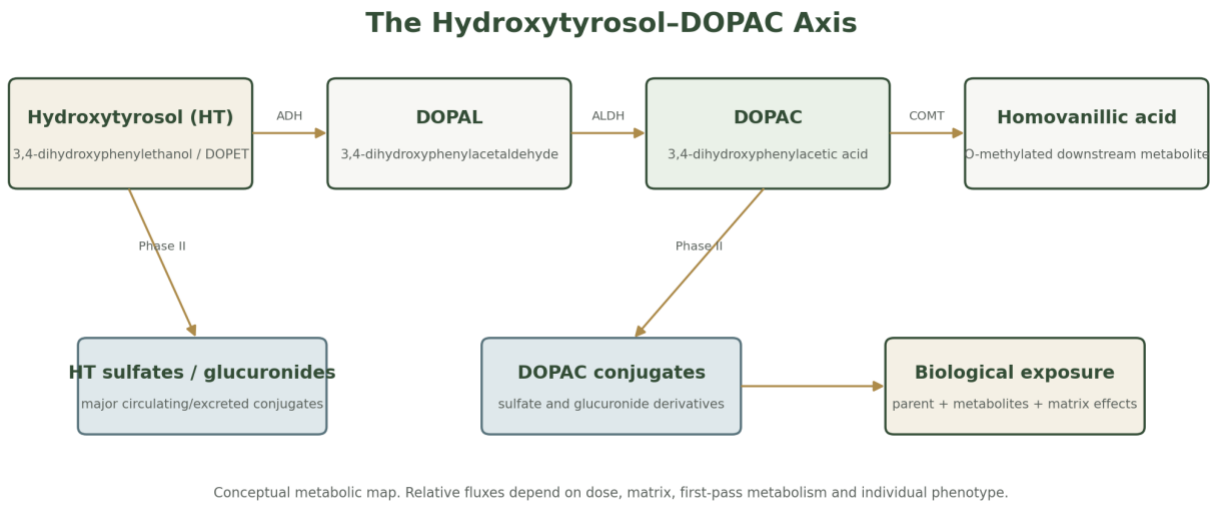

Figure 1. Conceptual Hydroxytyrosol–DOPAC metabolic axis. ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; COMT, catechol-O-methyltransferase. The diagram is interpretive and does not imply fixed quantitative fluxes.

5. The Hydroxytyrosol–DOPAC Axis

The most compelling reason to elevate DOPAC within HT research is quantitative human evidence. In a small controlled kinetic study after ingestion of ten Kalamata olives, plasma DOPET/HT rose substantially relative to baseline, but DOPAC rose much more: the reported DOPAC area under the curve was approximately 600-fold greater than the DOPET area under the curve. Because the olives themselves contained little DOPAC relative to HT, the investigators interpreted this as evidence of extensive in-vivo conversion of dietary HT to DOPAC. [7]

The biochemical route is plausible: oxidation of the terminal alcohol of HT can generate DOPAL, followed by aldehyde dehydrogenase-mediated formation of DOPAC. DOPAC can subsequently undergo methylation to homovanillic acid and can itself be conjugated. These reactions place DOPAC inside a metabolite network rather than at a dead-end.

Whether DOPAC contributes materially to human clinical outcomes remains unresolved. Experimental literature demonstrates that HT conjugates can retain biological activity in endothelial/inflammatory models, and newer work continues to examine DOPAC in neurobiological contexts. These data support the general proposition that metabolism does not necessarily equal inactivation, but they do not permit attribution of clinical HT effects specifically to DOPAC. [13,14]

A rational next-generation development programme would therefore measure both parent and metabolites and test whether clinical responders exhibit a distinctive HT:DOPAC:conjugate profile. This could distinguish true pharmacodynamic heterogeneity from simple variation in compliance or dose.

6. The 2025 Prediabetes Randomized Trial

Moratilla-Rivera and colleagues conducted a 16-week, parallel, randomized, double-blind, placebo-controlled trial in adults aged 40–70 years with overweight and prediabetes. Fifty-two participants were randomized and 49 completed the intervention; 24 received HT and 25 placebo. The active intervention supplied 15 mg HT/day. Oxidized LDL was the primary outcome; secondary outcomes covered biochemical/metabolic variables, oxidative-stress markers, inflammatory markers and lifestyle measures. [1]

The most important positive findings were not conventional glucose or lipid changes. The intervention improved oxidized LDL and produced favourable changes in protein carbonyls and 8-hydroxy-2′-deoxyguanosine, while total antioxidant status and glutathione peroxidase activity were better preserved. Interleukin-6 behaviour also differed between groups. These effects are coherent with modulation of oxidative and selected inflammatory processes.

Equally important are the negative findings. The study did not demonstrate clinically meaningful improvements in fasting glucose, HbA1c, insulin resistance indices, standard lipids, body weight or anthropometric outcomes. The trial therefore supports biological activity but not reversal of prediabetes, weight loss or conventional lipid lowering.

What this trial changes
It strengthens the human evidence that 15 mg/day of stand-alone HT can modify oxidative/inflammatory biology in an at-risk population over 16 weeks. It does not establish reduction of age-related disease incidence. That distinction should remain explicit in technical and marketing communication.

7. The 2026 Metabolomic Follow-Up

An untargeted LC–MS metabolomic analysis was subsequently conducted on serum from the same 49 participants. Compared with placebo, HT supplementation was associated with a distinct metabolic profile characterized by reduced nitrogenous-base derivatives and arachidonic acid, together with increases in selected phosphatidylcholines, lysophosphatidylcholines and sphingomyelins. Pathway analysis highlighted purine degradation and arachidonic-acid metabolism. [2]

This finding is mechanistically interesting because arachidonic-acid metabolism intersects with eicosanoid signalling and inflammation, while purine catabolism integrates nucleotide turnover and uric-acid/redox biology. Changes in phospholipid classes may reflect membrane and lipoprotein remodelling. However, untargeted metabolomics generates associations that require targeted validation, and the study is not an independent clinical replication because it reuses the original randomized cohort.

The metabolomic study should therefore be read as a mechanism-generating extension of the 2025 trial: it adds biochemical resolution to a clinical signal, but it does not add a second independent population.

8. What the Wider Human Evidence Shows

The broader human literature is encouraging but heterogeneous. Differences in population, matrix, duration, dose and co-ingredients are large enough that simple pooling by ‘milligrams of hydroxytyrosol’ can be misleading.

A 2017 randomized double-blind crossover trial in 28 generally healthy adults used 15 mg/day HT for three weeks and reported changes in several oxidative-stress variables and related gene-expression endpoints. Its short duration, multiple endpoints and healthy population limit direct extrapolation to disease prevention, but the study supports biological activity at the same nominal daily dose later used in the prediabetes trial. [15]

A six-month randomized study in 29 women with overweight/obesity compared 15 mg/day, 5 mg/day and placebo. It explored weight, fat mass and urinary metabolomics. The study is useful for duration and dose comparison but was small and was not designed as a definitive cardiometabolic outcome trial. [16]

In chronic coronary syndrome, a 2023 randomized crossover trial evaluated hydroxytyrosol-enriched olive oil providing 10 mg/day HT for one month. Improvements were reported in flow-mediated dilation, arterial stiffness, coronary flow reserve and several oxidative/inflammatory markers, with no effect on blood pressure. Because the intervention matrix was HT-enriched olive oil rather than purified HT, the results support the olive-phenol concept but cannot be assigned exclusively to free HT. [17]

Combination formulas add another evidentiary layer. A 2019 crossover trial of HT plus punicalagin (9.9 mg HT + 195 mg punicalagin) reported improvements in selected early atherosclerosis markers, while a 2022 analysis of an HT/punicalagin supplement found favourable changes in dyslipidaemic subgroups. These studies are relevant to formulation strategy, but attribution to HT alone is impossible because punicalagin is itself bioactive. [18,19]

Accordingly, the most defensible summary is that human evidence converges most consistently on oxidative-lipid biology and some vascular/inflammatory markers, while effects on body weight, glucose regulation, LDL-C and blood pressure are less consistent and strongly context dependent. The 2026 narrative review by Moratilla-Rivera et al. reaches a similar conclusion: clinical evidence remains limited and heterogeneous, and longer trials incorporating omics and interindividual variability are needed. [3]

9. Mechanistic Interpretation

9.1 Oxidative modification of lipoproteins

The most mature human signal concerns oxidation rather than concentration of LDL. This distinction is fundamental. A compound may alter susceptibility of lipoproteins to oxidative modification without materially lowering LDL-C. EFSA’s authorised olive-oil-polyphenol claim is itself framed around protection of blood lipids from oxidative stress, not LDL-C reduction.

9.2 Endogenous antioxidant defence

Changes in glutathione peroxidase, total antioxidant status and redox-responsive pathways are compatible with modulation of endogenous defence rather than simple stoichiometric radical scavenging. This is biologically plausible because the circulating concentrations of dietary phenols are generally too low to explain systemic effects exclusively by direct chemical quenching.

9.3 Inflammatory signalling

HT and its metabolites have been investigated in NF-κB-associated pathways, adhesion molecules and cytokine-related responses. Human trials show selected changes rather than uniform suppression of inflammation. The appropriate interpretation is pathway modulation, not a generalized anti-inflammatory drug effect.

9.4 Vascular function

Endothelial function, arterial stiffness and glycocalyx-related measures are attractive translational endpoints because they integrate oxidative and inflammatory burden. The 2023 coronary-disease crossover trial is supportive, but replication with standardized stand-alone HT and longer duration would materially strengthen inference.

9.5 Glucose homeostasis

Preclinical antidiabetic mechanisms cannot be treated as human efficacy. The 2025 prediabetes trial did not show improvement in the principal glycaemic measures despite oxidative/inflammatory changes. This may mean the dose/duration was insufficient, that the pathway does not translate into glucose lowering, or that HT acts upstream without producing short-term glycaemic effects.

9.6 Neurodegeneration and brain ageing

Olive phenols are actively studied in amyloid and α-synuclein biology, and DOPAC has particular relevance because of its relationship to dopamine metabolism. Current evidence in this area is predominantly preclinical. It is scientifically reasonable to discuss mechanistic research; it is not reasonable to imply demonstrated prevention or treatment of Parkinson's or Alzheimer's disease.

9.7 Microbiome and epigenetics

Emerging literature suggests interactions between olive phenols, microbial metabolism and epigenetic regulation. These areas are promising but not yet mature enough to support product-specific clinical conclusions. They are best used to design future biomarker programmes rather than current claims.

10. Formulation and Matrix Effects

The formulation problem is frequently underestimated. HT is polar and chemically reactive; absorption is rapid, while first-pass metabolism is extensive. The food matrix can change release, co-absorption, metabolic conversion and the relative exposure to parent versus conjugates.

- Free HT concentration and chemical purity.
- Contribution of oleuropein/secoiridoid precursors that may release HT during digestion.
- DOPAC already present in the raw material versus DOPAC generated metabolically after ingestion.
- Water activity, pH, oxygen exposure, metals and packaging, all of which can influence catechol stability.
- Lipid versus aqueous matrix and the possibility of different distribution/absorption kinetics.
- Release profile of capsules, gastro-resistant systems or microencapsulation.
- Co-phenolics and other actives that may create synergy but complicate causal interpretation.

For product development, the practical objective should therefore be reproducible biological exposure, not merely achievement of an assay value at release. Stability studies should monitor the phenolic profile over shelf life and under realistic accelerated conditions, with predefined acceptance limits for loss of HT and formation of relevant degradation products.

11. Analytical and Quality-Control Implications

For a high-quality HT or HT/DOPAC ingredient, a specification based only on 'total hydroxytyrosol' is insufficient. The analytical package should be constructed around identity, potency, related catechols, precursor phenolics, impurities, stability and contaminants.

A fit-for-purpose HPLC or UHPLC method with UV/diode-array detection can be appropriate for routine potency if specificity is demonstrated. LC–MS/MS is particularly valuable during method development, metabolite confirmation, complex matrices and investigation of degradation pathways. Reference standards and response factors should be appropriate to each analyte; 'HT equivalents' should not be confused with direct quantification of chemically distinct species.

Recommended development concept
Define two linked specifications: (1) a release specification for identity, assay, DOPAC/related phenols and contaminants; and (2) a stability-indicating profile that monitors HT loss and growth of predefined oxidation/metabolism-related markers. For clinical batches, add an expanded phenolic fingerprint to support batch bridging.

12. Dose: What Can and Cannot Be Inferred

The recent human literature repeatedly uses doses in the approximate 5–20 mg/day range, but this should not be converted into a universal 'effective dose'. Dose-response inference is weakened by small cohorts, different populations, matrices and endpoints. The 15 mg/day dose has now appeared in both short healthy-volunteer work and the 16-week prediabetes RCT, which makes it a useful research anchor, not a universally established optimum.

Similarly, the EU condition of at least 5 mg HT and derivatives per 20 g olive oil is a regulatory condition attached to a specific olive-oil-polyphenol claim. It is not a clinical minimum effective dose for isolated HT and not an automatic dose recommendation for food supplements. [8]

An evidence-driven dose programme should ideally compare at least two exposures, measure adherence and circulating/urinary metabolites, and pre-specify a mechanistic biomarker set. Without pharmacokinetic phenotyping, apparent dose non-response may simply reflect differences in absorption or first-pass conversion.

13. European Union Regulatory Position

Regulation (EC) No 1924/2006 governs nutrition and health claims in the EU, while Commission Regulation (EU) No 432/2012 lists authorised Article 13(1) claims. For olive-oil polyphenols, the authorised wording is that they contribute to the protection of blood lipids from oxidative stress. The claim may be used only for olive oil containing at least 5 mg hydroxytyrosol and its derivatives (for example, oleuropein complex and tyrosol) per 20 g olive oil, and consumers must be informed that the beneficial effect is obtained with a daily intake of 20 g olive oil. [8]

The wording and conditions matter. They do not create a generic claim for isolated HT, olive-leaf extracts, capsules or fortified foods merely because these contain 5 mg or more HT. Product category, ingredient status, authorised wording and conditions of use must all be assessed.

In May 2025, EFSA evaluated a proposed Article 14 disease-risk claim for phenolic compounds naturally present in olive oil, relating to lowering LDL-C and systolic blood pressure and thereby reducing coronary heart disease risk. EFSA concluded that a cause-and-effect relationship had not been established for reduction of LDL-C or systolic blood pressure. [9]

This recent negative opinion is highly relevant to communication strategy: oxidative-protection evidence should not be inflated into LDL-lowering, antihypertensive or coronary-disease-risk claims without a separate authorised basis.

14. Safety and Risk Assessment

EFSA's 2017 novel-food opinion assessed chemically synthesized hydroxytyrosol for specified uses in oils and fats. The Panel identified a NOAEL of 50 mg/kg body weight/day from a 90-day rat study and concluded that the novel food was safe under the proposed uses and use levels, considering margins of exposure and expected dietary intake. The target population in that assessment excluded children under 36 months, pregnant women and breastfeeding women. [20]

That opinion should not be interpreted as an unlimited safety endorsement for every source, dose or matrix. Novel-food specifications are ingredient-specific, and safety conclusions attach to the assessed material and

conditions of use. Separate consideration may be required for concentrated extracts with different impurity profiles, new manufacturing routes, substantially different exposures or deliberate HT/DOPAC combinations.

DOPAC itself also requires ingredient-specific regulatory and toxicological assessment if intentionally added rather than merely present as a metabolite or naturally occurring constituent. Metabolic familiarity is not, by itself, a regulatory authorization.

15. Research Agenda for Next-Generation HT/DOPAC Products

The field is now mature enough to move beyond generic antioxidant studies. A technically strong development programme would integrate chemistry, pharmacokinetics, systems biology and clinical outcomes.

- Pharmacokinetic mapping of HT, DOPAC, HT-sulfates, HT-glucuronides, DOPAC conjugates and homovanillic acid after standardized formulations.
- Direct comparison of purified HT versus an HT-rich olive matrix at matched HT-equivalent doses.
- Dose-ranging studies with pre-specified exposure-response modelling rather than post-hoc subgroup interpretation.
- Longer trials in prediabetes or metabolic syndrome with progression-related outcomes and vascular endpoints.
- Targeted validation of the 2026 metabolomic signals in arachidonic-acid and purine pathways.
- Integration of lipidomics, metabolomics and inflammatory proteomics to identify responder phenotypes.
- Characterization of gut-microbiome contribution to HT/DOPAC disposition and interindividual variability.
- Stability-indicating formulation work linking shelf-life chemistry with human exposure.
- Where scientifically and regulatorily appropriate, factorial evaluation of HT and DOPAC to determine whether deliberate co-exposure changes pharmacokinetics or pharmacodynamics.

High-value hypothesis
The most differentiating research question is not simply whether more HT is better. It is whether a reproducible HT–DOPAC metabolite profile predicts biological response better than the administered parent dose. That hypothesis is testable and could materially improve both formulation science and clinical stratification.

16. Conclusions

Hydroxytyrosol has moved beyond the stage at which its relevance can be justified only by in-vitro radical-scavenging assays. Controlled human studies demonstrate absorption, extensive metabolism and effects on several oxidative, inflammatory and vascular biomarkers. The 2025 randomized prediabetes trial is particularly important because it examined an at-risk population for 16 weeks and demonstrated favourable changes across multiple oxidative-stress markers at 15 mg/day.

The same study also sets necessary limits: glucose homeostasis, anthropometry and conventional lipid parameters did not materially improve. The evidence therefore supports biological modulation, not proven prevention or reversal of age-related chronic disease. The 2026 metabolomic extension adds plausible pathways but is mechanistic follow-up of the same cohort rather than independent replication.

Pharmacokinetics provides the second major conclusion. Human exposure after oral HT includes DOPAC and multiple phase-II metabolites, and DOPAC may become quantitatively prominent after olive ingestion. The scientifically useful unit is therefore a hydroxytyrosol metabolite network. Future products and trials that quantify this network are likely to be more informative than those that report only the nominal milligrams of HT administered.

For INTABIOTECH, historical technical experience with both HT and DOPAC creates a credible platform for this next step, particularly in manufacturing control, analytical fingerprinting and formulation design. The strongest positioning is not to overclaim present clinical evidence, but to develop a differentiated, metabolite-aware platform capable of generating the evidence the field currently lacks.

Appendix A. Selected Human Evidence Matrix

Study	Population / design	Intervention	Duration	Main signal	Key limitation
González-Santiago et al., 2010 [4]	Healthy volunteers; PK, n=10	Purified HT 2.5 mg/kg BW	Single dose	Rapid plasma appearance; urinary HT/HVA/DOPAC and conjugates; transient LDL association	High single dose; PK study, not efficacy trial
Mateos et al., 2016 [5]	Healthy adults; randomized double-blind crossover, n=13	HT-enriched biscuits, 5.25 mg	Acute	HT-sulfate and DOPAC-sulfate prominent; lower postprandial oxLDL	Acute feeding; food matrix
Colica et al., 2017 [15]	Healthy adults; randomized double-blind crossover, n=28	HT 15 mg/day	3 weeks	Changes in oxidative biomarkers and redox/inflammatory gene expression	Short duration; multiple endpoints; healthy population
Quirós-Fernández et al., 2019 [18]	Middle-aged adults; randomized placebo crossover, n=84	HT 9.9 mg + punicalagin 195 mg	8 weeks/period	FMD/oxLDL/BP improvements in selected abnormal subgroups	Combination product; subgroup findings cannot be attributed to HT alone
Fytili et al., 2022 [16]	Women with overweight/obesity; randomized double-blind, n=29	HT 15 mg/day, 5 mg/day or placebo	6 months	Exploratory body-composition and urinary-metabolomic effects	Small sample; not powered for hard cardiometabolic outcomes
Bender et al., 2023 [6]	Healthy volunteers; blinded crossover PK, n=12	Olive-derived supplements ~30.6 or 61.5 mg HT; fortified oil comparator	Single dose	Dose-related bioavailability; HVA, HT-3-sulfate and DOPAC major metabolites	PK only; relatively high acute doses
Ikonomidis et al., 2023 [17]	Chronic coronary syndrome; randomized double-blind crossover, n=30	HT-enriched olive oil, 10 mg HT/day	1 month/period	Improved FMD, PWV, coronary flow reserve; reduced oxLDL/MDA/CRP	Olive-oil matrix; short duration; small cohort
Moratilla-Rivera et al., 2025 [1]	Overweight + prediabetes; randomized double-blind parallel, 52 randomized/49 completed	HT 15 mg/day	16 weeks	Improved oxLDL, protein carbonyls, 8-OHdG; preserved antioxidant status; selected IL-6 effect	No material improvement in glycaemic indices, standard lipids or anthropometry
Moratilla-Rivera et al., 2026 [2]	Same 49 participants; untargeted serum metabolomics	Same HT 15 mg/day intervention	16 weeks	Signals in arachidonic-acid metabolism, purine degradation and phospholipid classes	Mechanistic re-analysis of same cohort, not independent replication

Appendix B. Suggested Analytical Specification Framework

Parameter	Rationale	Typical approach	Development note
Identity / HT assay	Confirm principal active and potency	HPLC/UHPLC-DAD; LC–MS confirmation	Use qualified reference standard and stability-indicating separation
DOPAC	Relevant metabolite/related catechol; differentiator for HT–DOPAC platform	HPLC/UHPLC; LC–MS/MS for specificity	Define whether native ingredient DOPAC is a controlled constituent, impurity or intentional component
Oleuropein / secoiridoid profile	Precursors may contribute to in-vivo HT exposure	HPLC-DAD/MS fingerprint	Important when comparing olive extracts with purified HT
Tyrosol / HVA / related phenols	Characterise co-phenolics and transformation products	Targeted HPLC/LC–MS	Set reporting or acceptance limits according to process
Water / water activity	Stability and microbiological relevance	Karl Fischer / aw meter	Link to accelerated and real-time stability
Oxidative degradation profile	Catechol compounds are oxidation-sensitive	Stability-indicating LC method	Monitor growth of critical degradation peaks
Residual solvents	Process control	GC-FID/GC-MS	Based on manufacturing route and ICH/food requirements
Heavy metals	Safety / regulatory quality	ICP-MS/OES	Define Pb, Cd, Hg, As limits appropriate to market
Pesticides	Botanical raw-material control	Multi-residue LC/GC-MS	Risk-based panel according to source and market
Microbiology	Food/supplement quality	Compendial food microbiology	Set category-appropriate limits
Batch fingerprint	Clinical and commercial comparability	Chromatographic fingerprint + key ratios	Retain for batch bridging and deviation investigations

Appendix C. Regulatory Communication Guardrails (EU)

Scientifically defensible in a review
Discuss human changes in oxLDL, oxidative DNA/protein markers, selected inflammatory variables, vascular measurements and metabolomic pathways, with the design limitations stated.

Requires caution
Language such as “supports healthy ageing”, “supports vascular resilience” or “helps maintain metabolic health” may still constitute a health claim depending on context and must be checked against the authorised claims framework before commercial use.

Not supported as a current stand-alone HT claim
“Prevents diabetes”, “reduces cardiovascular disease risk”, “lowers LDL cholesterol”, “lowers blood pressure”, “prevents neurodegeneration” or equivalent disease-treatment/prevention wording.

Authorised olive-oil context
“Olive oil polyphenols contribute to the protection of blood lipids from oxidative stress” only under the conditions in Regulation (EU) No 432/2012, including ≥5 mg HT and derivatives per 20 g olive oil and the required intake information.

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Scientific and Regulatory Note

This document is a scientific technical review and not medical advice, a clinical efficacy dossier or an authorization for commercial health claims. Product-specific regulatory assessment must consider ingredient identity, source, manufacturing process, intended use, target population, dose, applicable novel-food status and the exact commercial wording proposed in each jurisdiction.