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20.08.2026, Kraków

Review of the PhD Thesis by Magdalena Wit entitled "*Protein Kinase D2 in the regulation of lipid droplet metabolism in enterocytes of the small intestine*"

The thesis submitted for review was prepared under the supervision of Dr hab. Grzegorz Sumara, Professor at the Nencki Institute of Experimental Biology and Head of the Dioscuri Centre for Metabolic Diseases, Polish Academy of Sciences.

The thesis addresses the role of protein kinase D2 (PKD2) as a driver of lipid droplet (LD) turnover in the small intestine during the postprandial phase, studied both in a model of a single olive oil bolus and in a model of chronic lipid overload in mice fed a high-fat diet. This considerably extends the current view of PKD2 in lipid metabolism beyond its established role in chylomicron-mediated lipid transport. The role of LDs in fat absorption, and the possibility of modulating LD metabolism to protect against obesity, is an important direction in current research. The findings suggest that PKD2 is a promising target for pharmacological intervention aimed at limiting intestinal lipid absorption and thereby counteracting obesity. This is a question of growing importance, and I can state without hesitation that the thesis belongs to a current international research trend and addresses scientifically relevant problems.

The thesis contains a substantial body of independent experimental work and at least one original finding of genuine publication potential, namely the degradation of ATGL induced by dietary fat intake. This finding rests on a broad and well-considered set of models. The main phenotype is documented by several independent methods, and the level of methodological reflection in the Discussion is high.

On examining the thesis submitted for review in formal terms, it should be stated that it follows the layout typical of doctoral dissertations. The thesis opens with short abstracts written in English and in Polish, followed by a list of the abbreviations used. The subsequent parts are the introduction, the aims of the study, materials and methods, results, discussion, conclusions, and the bibliography.

The introduction is clear and well thought out. It guides the reader through all the aspects needed to understand the results and interpret the data. The figures are clear, well presented, and of high quality, and, as indicated in the captions, they are the candidate's own work.

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The aims of the study are stated clearly and precisely, and are presented after the theoretical introduction. The main aims were:

1. Determining the role of PKD2 in the regulation of LD metabolism and turnover in enterocytes of the small intestine;
2. Identifying the PKD2-dependent molecular mechanisms controlling LD dynamics in enterocytes;
3. Characterising the newly identified process of fat-induced degradation of lipid droplet-associated lipases (ATGL, HSL and CES2) and defining the role of PKD2 in its regulation.

The third aim is honestly presented as an unplanned finding identified in the course of the work. This is good practice and is not often seen.

The Materials and Methods section describes the techniques used in the study. It should be stressed that the methodology covered a wide range of approaches: mouse genotyping, *in vivo* experiments involving the administration of lipids, glucose, casein and other compounds, microscopy methods including immunohistochemistry and electron microscopy, organoid culture and cell culture, and lipidomic and proteomic analyses. Most procedures are described clearly and are easy to follow. I would, however, like to ask whether all techniques were performed by the candidate personally, or whether some of the more specialised ones, such as sample preparation for TEM and the imaging itself, were supported by a core facility, as is often the case. The results are presented appropriately, the descriptions are adequate, and the figures are clear and easy to interpret. The Discussion is detailed and well argued.

In terms of content, the thesis covers a broad set of questions. It examines the effect of PKD2 deficiency on the accumulation, formation and degradation of LDs in response to acute (olive oil gavage) and chronic (high-fat diet) lipid overload, and then asks how PKD2 influences the processes involved in LD turnover, including the regulation of catabolic pathways and the functional activity of lipolytic enzymes. The work also characterises the physiological significance of lipid-induced degradation of lipid droplet lipases and the cellular mechanisms behind this process.

The candidate builds a coherent mechanistic chain. Loss of PKD2 leads to disorganisation of the Golgi apparatus, which in turn impairs the delivery of ATGL to the LD surface, resulting in reduced lipolytic capacity and enlarged LDs. This chain is supported by transmission electron microscopy, immunofluorescence against PLIN2 and PLIN3, thin-layer chromatography, LC/MS lipidomics of isolated organelles, and subcellular fractionation. The fact that several independent methods point in the same direction is an important strength of the work, and it is what makes the main conclusion convincing.

The second part of the thesis, describing the rapid and selective degradation of ATGL and HSL after dietary fat intake, is in my view the most original element of the work. The candidate shows that this response is specific to lipids rather than to carbohydrates or protein, that it depends on the type of fat administered, that it is dose-dependent, and that it is prevented by blocking luminal lipolysis, chylomicron synthesis or triglyceride re-esterification. The observation that protein levels fall while the corresponding transcripts rise is a clear indication of post-translational regulation and is a finding of interest well beyond the biology of PKD2 itself.

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I regard the following as the outstanding strengths of the thesis:

1. A new physiological finding of potentially wide significance: rapid and selective degradation of ATGL and HSL in the intestinal epithelium after fat intake, accompanied by an opposite trend at the mRNA level, which points to post-translational regulation;
2. An unusually broad and well-planned set of models (two genetic mouse lines, acute and chronic lipid loading, apical-out, basal-out and 2D organoids, and Caco-2 cells). The apical-out and basal-out configurations were used deliberately to answer a specific mechanistic question, namely whether the route of fatty acid entry determines the susceptibility of ATGL to degradation;
3. Honest reporting of negative and inconsistent results. The Caco-2 model did not reproduce ATGL degradation, and oleic acid raised rather than lowered ATGL levels. The candidate states this openly, discusses a possible reason, and abandons the model instead of bending the interpretation. Similarly, Golgi–LD contact sites were not altered by the loss of PKD2, and the hypothesis of PKD2 acting as a tethering protein was rejected. Discrepancies between the Pkd2ki/ki and Pkd2gutΔ/Δ models in the lipase activity assay are also noted rather than passed over.

The thesis made for inspiring reading and raised several questions and comments:

1. After olive oil gavage, mice with intestine-specific PKD2 deficiency (*Pkd2gutΔ/Δ*) show both a higher number and a larger size of LDs in enterocytes than control mice (*Pkd2flox/flox*, *Fig. 11*), and at the same time they show more ATGL protein than controls (*Fig. 21*), with a comparable picture in the high-fat diet model (*Fig. 29/31*). As I understand it, the explanation offered is that ATGL is present but may not be correctly localised or fully active in the absence of PKD2. Could the PhD candidate comment on which of these two mechanisms is more likely?
2. The lipase activity assay did not show a significant reduction in the basal state in Pkd2gutΔ/Δ mice compared with controls (*Fig. 45*), although a significant reduction was reported for the Pkd2ki/ki line. The argument therefore rests mainly on the fact that Atglistatin lowered activity in control extracts but produced no further reduction in the knockout. A natural extension would be to repeat this assay after an olive oil challenge. A combination of more lipid droplets, ATGL protein preserved rather than degraded, and reduced activity under lipid loading would provide much more direct evidence. If such an experiment were feasible, what outcome would the candidate expect, and what hypothesis would she put forward?
3. Excess fat taken up during absorption can be directed either towards chylomicron synthesis or towards storage in lipid droplets. The Discussion states clearly that the present data cannot determine whether PKD2 governs this partitioning, and proposes selective depletion of lipid droplets, for example by silencing seipin, followed by measurement of chylomicron secretion. Could the candidate comment on how feasible this experiment would be, and what result she would expect?

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4. Could the candidate confirm how the DGAT inhibitors were administered? The Methods describe PF04620110 and PF07202954 as brought to 250 µL with sterile drinking water, while the legend to Fig. 27 states that they were injected intraperitoneally. The two routes differ in whether the compounds act locally in the intestine or systemically.
5. The composition of the high-fat diet is not given.
6. There is no table of antibodies. Since almost all the key conclusions of the thesis are based on Western blotting and immunofluorescence, information on the antibodies used, including supplier, catalogue number and dilution, would be needed for the work to be reproducible.
7. Scale bars are missing from several figures, or are not defined in the captions. More importantly, the captions do not consistently state what the individual points on the graphs represent, whether technical replicates, biological replicates or individual cells.

For a work of this size the number of typographical errors is small. The most important is that two different figures are both numbered Fig. 28, on pages 88 and 91. There are also a few incorrect cross-references, for example on p. 111, where the text refers to Fig. 30 A and B while the data are shown in Fig. 45 A and B, and in the caption of Fig. 45, where the quantification panels are given as B and C although B and D are meant. Placeholder citations marked "(ref)" remain in the text on pages 105 and 117.

However, the points mentioned above do not diminish the overall scientific value of the thesis. The quality of the results, the clear links between the different experiments, the detailed description of the research, and the use of appropriate methods to address the research questions demonstrate the candidate's ability to independently conduct and analyse scientific research.

I therefore state with great pleasure that the thesis submitted for review by Ms Magdalena Wit, entitled *"Protein Kinase D2 in the regulation of lipid droplet metabolism in enterocytes of the small intestine"*, fulfils all the conditions set out in Article 187, sections 1 and 2, of the Act on Higher Education and Science of 20 July 2018 (Dz.U. 2024 poz.1571). Therefore, I kindly ask the Scientific Council of the Institute of Experimental Biology to allow Magdalena Wit, to proceed to the next stages of the doctoral degree procedure. Given the high scientific level of the thesis, the methods applied and the value of the results obtained, I recommend that the thesis be awarded a distinction.

Marta Pacia

REVIEW OF THE DOCTORAL DISSERTATION

PhD Thesis Reviewer: Dr. Alison B. Kohan, Ph.D.
Associate Professor of Medicine
Department of Medicine
University of Pittsburgh
Pittsburgh, PA, USA

Name of Ph.D. student: Magdalena Wit

Affiliation: Dioscuri Centre for Metabolic Diseases at the Nencki Institute of Experimental Biology, Polish Academy of Sciences

Name of supervisor: Dr. Grzegorz Sumara, Ph.D., D.Sc.

Title of doctoral dissertation: Protein Kinase D2 in the regulation of lipid droplet metabolism in enterocytes of the small intestine

1. Scientific quality, data presentation, and organization of the dissertation

The dissertation moves in an orderly way from whole-body energy balance, through the anatomy and physiology of the small intestine, to lipid droplet biogenesis, protein trafficking to the droplet surface, lipolysis, and finally the protein kinase D family.

The quality of the data presentation is high. The transmission electron micrographs are excellent, and the candidate uses them quantitatively. Figure panels are well-labeled consistently, appropriate sample sizes and statistical tests are used throughout, and are also described in the legends. I especially like Fig. 8, which lays out the acute and chronic lipid challenge protocols, and Fig. 9, which explains the organoid system. The summary and conclusions are an appropriate synthesis of the presented data and hypotheses and is very thorough and clear.

2. The aims of the project

The central hypothesis of this thesis is that PKD2 may be a regulator of lipid storage and secretion in enterocytes. The aims follow logically and are fundamentally based on the Sumara laboratory's earlier demonstration that intestinal PKD2 promotes chylomicron-mediated fat absorption, possibly through phosphorylation of APOA4, and that its genetic or pharmacological inactivation reduces triglyceride output and protects mice from diet-induced obesity. Three aims test the central hypothesis: (1) Aim 1 proposed to determine the role of PKD2 in the turnover of intestinal LDs formed in response to both acute (via olive oil gavage) and prolonged dietary lipid load (through feeding with a high-fat diet); (2) Aim 2 proposed to elucidate the PKD2-dependent molecular mechanisms regulating LD dynamics; and (3) an additional objective was defined during the thesis period which proposed to characterize the previously unreported physiological process and to define the role of PKD2 in its regulation. I appreciate the additional information about Aim 3, which arose during the thesis work and is a novel addition to the field's understanding of enterocyte lipid trafficking and chylomicron synthesis and secretion.

The premise for these aims is strong, and the approaches utilize the existing strengths of the Sumara Lab and extend these with a new model of primary intestinal organoids (developed by the thesis candidate). How the enterocyte apportions absorbed fat between immediate export and cytosolic storage is, in my view, a central unresolved question in intestinal lipid biology. This thesis is a strong addition to this gap in knowledge.

3. The employed research methodology

The candidate relies on a mix of *in vitro* and *in vivo* models, including an intestine-specific deletion of PKD2, and a whole-body kinase-dead knock-in. Using these models, the thesis clearly shows enlargement of lipid droplets (Fig. 11, Fig. 19), making the conclusions rigorous.

The *in vitro* work is comprised of three-dimensional organoids, apical-out organoids, two-dimensional monolayers derived from three-dimensional organoids, and Caco-2 cells carrying stable shRNA knockdown. My laboratory has also employed primary murine organoids for studies of dietary lipid absorption (Jattan et al., JLR 2017) because we found transformed lines unsatisfactory for studies of fat absorption. I read the organoid work described here with both interest and sympathy for the technical difficulty involved. It is well-executed, and the apical-out approach is both clearly developed and used appropriately for luminal lipid delivery. I am excited to share the apical out procedure with my own lab as it is a very nice model that the thesis candidate has developed.

Pharmacological approaches are also used to establish that ATGL loss responds to dietary fat rather than to caloric repletion. Isocaloric comparisons of olive oil, linseed oil and coconut oil, suggest that medium-chain fat (which is not absorbed through the chylomicron synthetic pathway) does not trigger the observed changes to lipid droplet activity, while monounsaturated and polyunsaturated oils retain this capability. This is strong evidence for a relationship between the chylomicron synthetic pathway and ATGL/lipid droplet dynamics. The thesis employs gold-standard inhibitors of each step of the pathway including blockade of luminal hydrolysis with Orlistat, blockade of chylomicron assembly with Pluronic L-81, and blockade of triglyceride re-esterification with DGAT1 and DGAT2 inhibitors.

All experiments were carried out in male mice, which is common in the field, and the thesis clearly acknowledges the limitation of focusing on a single sex, given the possibility of sex differences in intestinal lipid handling and in response to high-fat feeding. This does not detract from the findings, nor from the central conclusions and is a practical aspect of intestinal research in animal models.

4. The presented results

The core observation is that enterocytes lacking functional PKD2 accumulate larger cytosolic lipid droplets after an acute olive oil gavage, the droplets fail to normalize over an extended post-prandial period, and the same phenotype appears under chronic high-fat feeding but not during fasting. The lipidomic analysis by organellar fraction is carefully done and suggests that triglyceride accumulates in the droplet fraction rather than across membranes generally. Subcellular fractionation shows that ATGL association with the lipid droplet fraction is abolished in the absence of PKD2. Electron microscopy and immunofluorescence show disorganized Golgi cisternae in PKD2-deficient enterocytes and in PKD2-knockdown Caco-2 cells. Since ATGL delivery to the droplet surface depends on early secretory trafficking machinery, the conclusion that the Golgi is the site of the block is reasonable and supported by these data and the literature. Additionally, this thesis finds that proteolysis of ATGL in response to dietary fat occurs during fasting-refeeding with high-fat but not a fat-free diet, and in organoids treated with oleic acid. Proteomics then show broad remodeling of the enterocyte proteome after lipid ingestion.

5. The presented conclusions

The candidate concludes that PKD2 is a determinant of lipid droplet turnover in enterocytes, that it acts at least in part by permitting ATGL delivery from the Golgi to the lipid droplet surface, and that dietary fat ingestion triggers a physiological degradation of ATGL, which is prevented by PKD2 deficiency. These conclusions are adequately placed in context throughout the discussion, which explicitly states that these data cannot establish whether PKD2 governs the partitioning of absorbed fat between secretion and storage pathways, and further, that the chylomicron and lipid droplet phenotypes of PKD2 depletion may reflect two independent functions of the kinase. This is a very mature analysis and points to the next logical set of studies.

6. The discussion

The discussion is thorough, and the candidate is specific about potential alternatives to both methodological choices and to the conclusions drawn. This is very well done. Further, the candidate has identified mechanistic experiments that would extend her current findings; for example, Seipin silencing and the separation of triglyceride incorporated into chylomicrons from triglyceride derived from lipid droplets and the role of PKD2 in this process. The candidate acknowledges that HSL, MGL, CES2 and LAL expression and activity were not measured, and may deserve future attention. The candidate notes that lipid droplet biogenesis rates were assessed only by transcript abundance and proposes the LiveDrop reporter as a better approach [this was my first time learning about LiveDrop, and I agree that this is a very interesting approach!]. The candidate observes that her *in vitro* lipase assay occurs outside any organellar context, since all membranes are solubilized, and this may obscure a potential source of regulation between organelles that was not captured. The section on the physiological purpose of ATGL degradation is appropriately speculative, and the candidate hypothesizes that acute lipase removal may function to limit the cytosolic free fatty acid burden during the postprandial period, where there is high luminal fatty acid; this would limit potential lipotoxicity. These points are well-stated and mature throughout and add significantly to the overall work.

7. The choice of literature cited

The bibliography is appropriate in scope and content. The lipid droplet literature is well represented, as is the intestinal lipid absorption literature, including the foundational work on chylomicron formation and secretion. Recent work is cited alongside primary sources.

8. Detected mistakes, shortcomings, and inaccurate wording

The scientific content is sound and represents a novel addition to our field's understanding of lipid trafficking in the enterocyte (between the lipid droplet and the chylomicron synthesis pathways) and its effect on dietary fat absorption properties. The candidate should be commended for her use of unique models across *in vitro* and *in vivo* studies, as well as working within a tough tissue to mechanistically dissect.

Some copy-editing notes, none of which are significant nor detract from the quality of the science:

- I noticed two citation placeholders "(ref)" (p. 105 and p. 117) that should be updated prior to publishing the final draft.
- Bafilomycin dose is written as both 0.1 mg/kg body weight (methods), and 100 μg/g body weight (figure legend). This could be synchronized.
- Check: "complimentary" versus "complementary"; "performer" versus "performed"; "manager" versus "managed"; "hipertriglycerydemia" and "hipercholesterolemia" versus "hypertriglyceridemia" and "hypercholesterolemia".
- The mechanism of Pluronic L-81 is described as inhibition of MTTP function. L-81 does block the maturation and transport of pre-chylomicron particles from the endoplasmic reticulum, producing accumulation of small dense particles, but the attribution of this effect specifically to MTTP inhibition may not be fully settled science. The candidate may want to consider softening that language, but it's not required. If I am wrong about the L-81 mechanism (entirely possible!), please disregard.

9. Does the dissertation provide an original solution to a scientific problem?

This dissertation positively identifies an important gap in knowledge in our field, develops the appropriate models to explore that gap, and then identifies new mechanisms which bring the field closer to filling the fap. This dissertation finds: (1) that PKD2 is as a regulator of lipid droplet catabolism in the enterocyte, acting via the delivery of ATGL from the Golgi to the droplet surface. This extends the laboratory's previous work on PKD2 and chylomicron secretion, and (2) that inhibition of ATGL is dependent on long chain fat absorption and may be central to partitioning dietary fat between pathways.

Both findings exceed the standard of an original solution to a scientific problem.

10. Does the dissertation demonstrate general theoretical knowledge and the ability to conduct independent research?

The introduction demonstrates command of a literature spanning whole-body energy metabolism, intestinal physiology, lipid droplet biology, and membrane trafficking. Assembling these into a coherent narrative requires a strong command of the literature and identification of knowledge gaps. The candidate has demonstrated skill at both throughout the document.

The candidate's publication record also supports this assessment. The candidate is first author on two review articles, in *EMBO Molecular Medicine* and in *Biochimica et Biophysica Acta*, both of which address the subject matter of this thesis directly, and a co-author on two experimental papers in EMBO Molecular Medicine.

In my opinion, the strongest evidence of the independent scientific judgment of this candidate is her written discussion. The candidate identifies the limitations of her own data and for each limitation she proposes an experimental remedy. This is both thoughtful and mature and demonstrates both general theoretical knowledge and a strong ability to conduct independent analysis and research.

11. Questions and points for discussion at the defense

- The laboratory's earlier work proposed that PKD2 promotes chylomicron size, at least in part through phosphorylation of APOA4, and that PKD2 inhibition/loss reduces particle size and triglyceride secretion. The Tso Lab previously showed, in apoA-IV knockout mice, that the loss of apoA-IV resulted in larger chylomicrons that were cleared from plasma more slowly, with no gross defect in absorption (Tso et al., AJP 2012; 2013). However, the apoA-IV field is still not fully convinced of the role of apoA-IV in chylomicron synthesis and secretion. Is it possible that PKD2 is the “missing link” that could explain why different labs have attributed different roles to intestinal apoA-IV?
- Fig. 13 shows chylomicrons stacked in the intercellular space and lamina propria. Does the candidate interpret this as failure of the secreted chylomicrons to enter the lacteal, or reduced basolateral exo- or endocytosis, or could there be a change in chylomicron re-uptake by the enterocyte?
- Pluronic L-81 pretreatment prevents ATGL degradation in the intestine. Does the candidate believe chylomicron assembly is required for the ATGL degradation signal (and that L-81 blocks this signal) or is it possible that L-81 alters the intracellular fate of absorbed fatty acids upstream of chylomicron assembly?
- Both proteasomal and lysosomal inhibition rescue ATGL. Does the candidate have any sense that one might be more important than the other during fat absorption and chylomicron secretion? Is this testable?
- If ATGL degradation is protective against cytosolic fatty acid overload, what would the candidate predict for intestinal barrier function and epithelial turnover in an animal expressing degradation-resistant ATGL under chronic high-fat feeding?
- A general question. If the Golgi is the coordinating point for dietary lipids to “choose” a route of lipid droplet storage or export via chylomicrons as this thesis suggests, how could this be tested in future experiments?

12. Overall evaluation

This is an outstanding thesis. The question it addresses is central to the field. The experimental design is rigorous, and the discussion places the work within the context of the current field.

The dissertation complies with requirements listed in Art. 187 Sections 1 and 2 of the Act of July 20, 2018 - Law on Higher Education and Science (Journal of Laws 2018 item 1668 as amended).

I therefore recommend that Ms. Magdalena Wit be admitted to the subsequent stages of the doctoral defense.

13. Application for distinction of the dissertation

I recommend that this dissertation be distinguished, and I ask that this application be recorded in the written review as required. This nomination is based on several points: (1) the thesis reports the discovery of a previously undescribed physiological process, and this work has resulted in multiple high-quality publications. (2) the rigorous application of controls is of a very high standard and is very well-described. (3) the use of complementary models and both *in vivo* and *in vitro* approaches is note-worthy. Two mouse lines, multiple primary organoid models, and pharmacologic inhibition and a knockdown cell line are all used. This is highly rigorous and exceptionally difficult molecular and physiological work. For these reasons I ask the Scientific Council to consider this dissertation for distinction.

Respectfully submitted,

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Alison B. Kohan, Ph.D.
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University of Pittsburgh
Pittsburgh, PA USA

Date: August 24, 2026

AI Usage Disclosure: AI was used to scan my written document for typographical errors and for readability cues. None of the content nor opinions expressed here are generated by AI and they are based solely my own expertise and work. I take full responsibility for the final content.

Jan-Wilhelm Kornfeld, Dr. rer. nat.
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ADIPOSIGN Novo Nordisk Foundation Centre for Adipocyte Signaling
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Re: Evaluation PhD thesis M.s Magdalena Wit:

Dear PhD committee of the Nencki Institute of Experimental Biology, Polish Academy of Sciences

Ms Magdalena Wit submitted her PhD thesis entitled: "*Protein Kinase D2 in the regulation of lipid droplet metabolism in enterocytes of the small intestine*" which was carried out at the Dioscuri Centre for Metabolic Diseases (Nencki Institute of Experimental Biology, Polish Academy of Sciences) under the supervision of Assoc. Prof Grzegorz Sumara.

Summary of the thesis:
Thematically, the work covers a large spectrum of methodologies ranging form in vivo work using PKD2-deficient/inactivated mice, intestinal organoids and cell lines, bioimaging, proteomics and expression profiling to delineate that protein kinase D2 (PKD2) serves key functions in enterocyte-mediated lipid handlings and lipid droplet (LD) formation. Starting from the seminal observation that various – but not all - high-lipid supplementation paradigms like acute olive oil and more chronic HFD feeding activate PKD2 which suppresses LD formation. The key observation prompting the conducted work was that PKD2 loss in enterocytes prevented the loss of lipid oxidation by stabilization of (otherwise proteosomally) degraded lipases such as HSL and ATGL, key factors initiating factors in LD-associated lipid degradation pathway. Although the order of findings presented was unorthodoxical - I would have started with a stronger rationale for choosing PKD2 or the observation that PKD2 is phosphorylated under high-lipid/lipotoxic conditions - Magdalena convincingly showed that conditional PKD2 KO (PKD2Δgut) and/or knock-in of a enzymatically inactivate PKD2KI variant causes atypically large LD/TAG accumulation which coincides with stabilization of the LD-degrading HSL/ATGL lipases, a seminal discovery which broad applicability to many conditions of acute lipid handling and lipotoxicity which might transcend the findings made in intestinal cells. Magdalena then shows that this process of ATGL degradation incurs the UPS / autophagy pathway, reproduces these findings in an organotypic (human) organoid system and conducts subcellular ATGL trafficking and lipase activity measurements to demonstrate that PKD2 is involved in ATGL-LD stabilization. She also reports a general effect of PKD2 loss on (trans)-Golgi formation which might contribute to the impaired subcellular trafficking and compartment interaction that she demonstrates and supports using TEM experiments. The overall quality of data is very high, appropriate controls are included and the breadth of methods applied to consolidate major findings is impressive.

Organization of the dissertation:
The thesis covers 149 pages including references and supporting pages and starts with an extensive *Introduction* that covers a broad palette of topics ranging from the physiological and anatomic basics of lipid metabolism in the gastrointestinal tract, the functional contribution of various intestinal cell types, lipid transport within and across cells, a very detailed and well-written description of LD formation and dynamics and the molecular processes and cellular compartments involved in turnover of proteins involves in LD regulation. The *Aims* section argues for a seminal role for PKD2 in intestinal LD based on earlier observations of PKD2 controlling lipoprotein/CM formation. Here the author could have highlighted the observation of PKD2 activation as foundational basis for conducting these experiments to avoid an impression of PKD2 being chosen due to topical reasons. Despite this observation

likely being made later in her thesis, it would have allowed a more physiology-driven rational for studying PKD2 and I would have encouraged a *Hypothesis* section as part of the aims to demonstrate independent, prospective thinking before embarking on the experiments. I would also like to discuss the choice of models used for which experiments (PKD2Δgut PKD2KI/KI; mice versus organoids versus Caco2 cells). The *Materials and Methods* section is of sufficient detail and presents with an impression breadth of methods, analytical workflows and modelling systems (mice, organoids, cells) which are not trivial to master, particularly in combination and given the inconsistencies that will arise from validation experiments in orthogonal systems. Statistical tests are appropriate although the choice of test not justified. I also missed information about the number of independent experiments with similar outcomes which would have helped underscore the robustness of findings, particularly for the data presented with low n (Western Blot, IF). It would also have helped to understand which parts of the methodology and analysis was done by the PhD candidate and what was carried in collaboration with a core facility (e.g., omics experiments, TEM). The *Results* section is again a highlight of the thesis: Magdalena presents a substantial body of data to support her claims which is certainly above-average for a PhD thesis. Most experiments are sufficiently powered (EM, mouse cohorts) whereas others would have profited from more replicates and/or information on reproducibility (lipase assays, WB). The omics assays would have profited from additional bioinformatics analysis like PCA/ gene clustering or lipid species modelling to understand group-level (size) effects. The author is encouraged to display individual data points to show variance across methodologies and/or show data as box-whisker plots instead of dynamite bars. A couple of misattributed figure references and typographies could also have been avoided by spell checking, particularly at the end of the dissertation. In summary though, the degree of novel insight, broad choice of methods and biological impact of data presented is exemplary and points towards a fundamentally physiological / cell biological mechanism which Magdalena should be highly commended for. The *Discussion and Summary* provides a thoughtful, yet in-depth, critical reflection of the data obtained that is paired with a highly creative plan for upcoming experiments and model systems (e.g., mouse model of degradation-resistant ATGL animal model) that will be insightful to discuss at the defense.

Evaluation of the scientific content:
Collectively, the PhD project aims to uncover new cellular and molecular mechanisms relevant to a physiologically highly relevant, understudied process, namely intestinal lipid handling and LD dynamics and employ a broad, controlled and orthogonal methodology to do so. The results are intriguing and speak for a broad relevance of mechanisms of ATGL/LD regulation that might transcend the gut and/or regulation by PKD2. The data obtained are at an impressive scale for a PhD thesis, the conclusions are appropriate, avoid overinterpretation and employ sound statistical testing to support the claims. The discussion is evidence of high rigor and creativity, reflecting scientific maturity and capacity for self-reflection. The literature is appropriately cited and allow to juxtapose against the findings made. No obvious mistakes/shortcomings, errors and wrong or inaccurate wording was observed, and the PhD dissertation provides an original solution to a scientific problem. The doctoral dissertation demonstrates the Ph.D. student's theoretical knowledge of the field and ability to conduct independent scientific work, and I assume that the findings made by the student will be published soon. In addition to her own work, Ms. Wit has contributed to two other papers in EMBO Mol Med (2021, 2023) as co-author and has written two reviews on intestinal lipid handling and PKD2 as first-author.

My combined evaluation of the submitted PhD work is outrightly positive. I confirm that the doctoral dissertation of Magdalena Wit entitled "*Protein Kinase D2 in the regulation of lipid droplet metabolism in enterocytes of the small intestine*" fulfills the requirements set out in the requirements listed in Art. 187 Sections 1 and 2 of the Act of July 20, 2018 - Law on Higher Education and Science (Journal of Laws 2018 item 1668 as amended)"

Given the quality of the presented thesis, **I would like to nominate Magdalena Wit's dissertation for distinction.**
With best regards,

(Jan-Wilhelm Kornfeld)