Valorization
Socioeconomic relevance

It is well recognized that diet and physical inactivity are major contributors to obesity and diabetes. Diabetes is one of the most common chronic diseases in the Netherlands, a disease that is for instance correlated with increased risk for cardiovascular disease. Especially for people with type 2 diabetes (T2D), regular exercise together with moderate caloric intake are important for maintaining health. Nevertheless, some people with T2D may also need diabetes medications, alternative therapies, dietary compounds or insulin therapy.

Epidemiological research shows that in the Netherlands around 1 million people have diabetes, with an incidence of 50.000 new cases a year. Approximately 3.12 billion euro is spent for taking care of diabetes patients, which is approximately 9% of the total health care costs (1-3). According to the World Health Organization, 350 million people suffer from diabetes, a number that may be doubled by 2025, and 90% of all diabetic patients have T2D (4). Because T2D affect an enormous amount of people, (e.g., due to financial and intangible costs), it is of great importance to arrest this growing global epidemic and improve the quality of life of T2D patients. Thus in an era of epidemic obesity, diabetes and sedentary lifestyles, there is serious need for effective and potentially curative therapies.

The dysregulation of AMPK plays an important role in the development of T2D and activation of AMPK (i.e., pharmacological or physiological) can prevent or ameliorate some of the pathologies. Nevertheless, the actions of activated AMPK have generated questions regarding its potential role at specific subcellular localizations such as glycogen. We know that activated AMPK improves glucose uptake, glycolysis and fatty acid oxidation to provide ATP, but what exactly occurs with and/or at glycogen, to our knowledge, has never been considered. Because phosphorylation of muscle glycogen synthase is known to inhibit the rate of glycogen synthesis, the present study aimed at investigating the molecular mechanism involved in the ‘cycling’ of AMPK to and from glycogen. We were excited to dissect a relatively unexplored area, the role and/or activity of glycogen-localized AMPK, with particular interest in glycogen metabolism, and employed a variety of biochemical, cell-biological and mutational studies. With the work described in this thesis, we envisage the novel concept that ‘cycling’ of AMPK might be another way of exercise to restore glycogen deficiency.

Our results can be considered as the first step towards clinical implementation (‘Bench to Bedside’). In a nutshell, we have shown the importance of the threonine-148 residue (Thr-148). Our data revealed that phosphorylation of this residue, both in cell-free assays and in vitro, prevents AMPK from binding to glycogen (5). Further investigation confirmed these findings, but also led to new mechanistic insight. The fact that the interaction between AMPK and other glycogen-binding proteins, such as the PP1 glycogen-targeting subunit R6 or glycogen synthase is dynamically regulated by glycogen, suggests a tight regulation of glycogen
metabolism, likely depending on the formation/collapse of an AMPK/R6/PP1 multiprotein complex. In order to decode the circuits linking fundamental cell biological processes to physiology, we further explored the role of ‘cycling’ of AMPK away from glycogen in cells that were made insulin resistant. Our data revealed that cytosolic AMPK was more active, had increased basal glycogen levels, enhanced glucose uptake, and was capable of preventing cells from becoming insulin resistant. Overall, the research described in this thesis presents a novel molecular mechanism that provides possibilities of utilizing it to combat T2D, which will be further elucidated below.

To gain insight into the possible clinical impact of shifting AMPK away from glycogen either on a healthy volunteer or on a T2D patient, the very first step to be undertaken is to translate the results presented into experimental animal models: investigate the in vivo (and/or ex vivo) regulation of AMPK, aiming at activity, subcellular localization, protein interaction and its role on glycogen metabolism. In other words, the question whether the ‘cycling’ of AMPK away from glycogen (by modifying the Thr-148 residue) does affect insulin-independent glucose uptake, and in particular intracellular glycogen levels, requires further investigation. The use of healthy rodent models, in combination with applying the toolbox and techniques established in the lab will provide detailed insight into the AMPK signaling cascade on transcriptional and translational level, intracellular localization, and the regulation of glycogen metabolism. Also studying important processes such as autophagy/glycophagy, fatty acid oxidation and gluconeogenesis will contribute to knowledge of the function of localized AMPK on whole-body level.

Although addressing this kind of questions will certainly lead to novel insights mimicking physiological situations, many clinically relevant questions would still remain unanswered. Since AMPK is rapidly activated during exercise/contraction, caloric restriction, or in response to pharmacological agents (e.g., metformin) sustained cytoplasmic AMPK activation may prove beneficial. However, does the shift of AMPK away from glycogen lower blood glucose levels in T2D conditions? Does it prevent the possible inhibitory effect of AMPK on glycogen synthesis? Or, alternatively, does it inactivate cytoplasmic glycogen synthase and thus negatively affect glycogen synthesis? The use of established disease models (e.g., diabetic ob/ob mice) would allow for addressing such kinds of questions and would ultimately help in understanding whether the deficiency in glycogen-storage observed in heart and skeletal muscle of T2D patients is preventable and/or reversible by modulating the subcellular localization of AMPK. Today it is the challenge of the scientist, in collaboration with different target groups, to find these answers, explain them to the patient, and translate them into the clinic.
**Target groups**

The involvement of patients, healthcare providers and society, *i.e.*, target groups, is fundamental to the quality care for metabolic diseases such as T2D. From a scientific point of view, this list of people/groups, being interested in our work or being important for knowledge development, can be further extended for instance with other scientists and/or collaboration partners, patient associations, foundations, and pharmaceutical industry.

To make other scientists aware of our recent discoveries, and also importantly, to gather novel insights from different research angles, thereby facilitating scientific progress and contribution, the studies described in this thesis were presented at relevant scientific conferences by means of oral presentations at national (Genetics Retreat, Rolduc, Kerkrade, the Netherlands, 2012; NVDO Annual Dutch Diabetes Research Meeting, Oosterbeek, the Netherlands, 2013) and international conferences (1st European AMPK Workshop, Maastricht, the Netherlands, 2013; Society for Heart and Vascular Metabolism (SHVM) Conference in Tromsø, Norway, 2014; FASEB Science Research Conference on AMPK, Lucca, Italy, 2014) and poster presentations (FASEB Science Research Conference on AMPK, Asilomar, Pacific Grove, California, 2012; 2nd European AMPK Workshop, Maastricht, the Netherlands, 2015). In addition, our findings were published in scientific journals relevant to the research field (*Journal of Biological Chemistry*). In the near future, it might be of interest to participate in and present our current knowledge on conferences such as the World Congress on Interventional Therapies for Type 2 Diabetes, as it is a comprehensive, multidisciplinary forum of worldwide specialists whose aim is discuss on research priorities and health policy initiatives, and gain further insight into how the study of increasing intracellular glycogen content may improve our understanding of T2D and provide direction for future treatments of curative intent.

To further encourage knowledge utilization and development, there is strong interest for collaboration with people directly connected to our research/the field; in other words, networking with current collaborators or colleague scientists that are willing to cooperate and/or provide for instance relevant mouse models related to our research. Further, approaching gene targeting companies (for example, GenoWay), as they may be involved in the production of highly physiologically relevant transgenic mouse models (*e.g.*, wild-type AMPKβ2 vs. AMPKβ2-T148D transgenic mice) might be of serious interest. In future, such mice may be used to explore the above mentioned physiological impact of Thr-148 phosphorylation on glucose and glycogen metabolism.
In the era of advanced therapeutic approaches, we established a new screening procedure for the identification of small molecules specifically targeting the carbohydrate-binding module (CBM) of AMPK. Based on this strategy, we aimed at shifting AMPK away from glycogen by means of small molecules, for instance, to modulate cardiac and/or skeletal muscle glycogen content. Besides targeting AMPK to prevent it from binding to glycogen, the top candidate molecule has been shown to activate the AMPK signaling pathway and induce nutrient (i.e., glucose and long-chain fatty acid) uptake. However, up until now, there was no isoform-specificity found (AMPKβ1 vs. AMPKβ2), underlining that optimization (e.g., chemical modification) is necessary to obtain isoform-specificity. Thus, the manufacturing of innovative molecules/drugs for clinical use must involve our pharmaceutical friends.

In order to make sure that innovative findings and discoveries result into improvements for patients, investment must be put in big national and international collaborations. In essence, one should keep in mind that it is the total assembly of knowledge, from scientists, clinicians, health care providers, human subjects, pharmaceutical industry and foundations that creates the value. Therefore, we will need to extensively discuss, interact and collaborate in order to valorize. An example of such national collaboration is the CVON (Dutch Cardiovascular Research), which supports large-scale national research projects in which researchers, universities, and industry work together. Being involved in grants such as CVON would provide an excellent opportunity for further knowledge development (6). Moreover, European grants such as Horizon2020 Projects or European Framework for Research Program should create and further support such international collaborations and sharing of expertise (7).

Activities and products
Products of great relevance for the society may be the awareness of diabetes as an epidemic. To make the knowledge described in this thesis more transparent, accessible and useful for the benefit of the society, one of the best target groups to reach would be foundations such as the Diabetes Fonds or Hartsstichting (1,6). Their function is to improve access to medicines and healthcare by disseminating knowledge to all layers of the population, making use of brochures and campaigns, and providing education, counseling and care. One of the aims/challenges could be to get more connected to such organizations. By giving them regularly comprehensible updates on the status/progress of the research, the transfer of knowledge to society will surely be facilitated and thus increased. In such way, the society (including the patient, relatives, generally interested people, contributors to the foundation, etc.) might better understand, realize, discuss or wonder about what kind of research (stepwise) is conducted in the lab for the benefit of the patient. Thus, by providing scientifically relevant information regarding the syndrome, which is intelligible for layman, we may create opportunities for improving the health status and quality of
life of the patient. Further, the organization Hart&Vaatgroep is an important association for patients or their relatives, which organizes regional or national meetings, workshops, courses and mutual patient connection (6). Making such organizations aware of the scientifically/clinically relevant questions being addressed will foster the exchange of knowledge for the benefit of the patient.

One of the products of great importance for future is based on the drug-targeted strategy presented in this thesis and thus may include novel molecules/drugs. After optimization of the top candidate molecule(s), the most potent molecule(s) would need to undergo pharmacodynamics, pharmacokinetics, ADME and toxicity testing through in vivo approaches before going into clinical trials. Although on average, only one in every 5,000 compounds that makes it through lead development to the stage of pre-clinical development becomes an approved drug these steps are of high value, thus determining the safest dose for first-in-human studies and evaluating the product’s safety profile. Ultimately, the drug should have selective capacity to shift AMPK away from glycogen, and similar safety and bioavailability as for instance metformin does. Ideally, the drug should have the capacity of stimulating glucose uptake, and finally, it should not cross the blood brain barrier to prevent influence on hypothalamic AMPK.

**Innovation and future directions**

It is of vital importance to invest in patient care and prevention, and thus in the pioneering research that is underlying, also to reduce for instance the risk of heart failure. In other words, identification of the molecular mechanisms involved in the onset and/or progression of the metabolic dysregulation ultimately allows for new insights and therapeutic developments for the regression and cure of T2D, and this is where our fundamental molecular-biology-oriented research contributes. Obviously, we do not provide here any evidence for a potential new medication that would lead to the cure of the type 2 diabetic patient. However, interestingly, this thesis provides important knowledge and state-of-the-art techniques that is needed as basis for the development of therapeutic strategies and/or techniques that may lead to novel treatment options for T2D. As described in the paragraphs above, our findings provide ample of possible opportunities (i.e., further exploring the novel drug-targeting approach, translational research by means of animal experimentation, pre-clinical testing, etc.) not only to address the importance of AMPK as metabolic master switch of the cell, but also to emphasize the relevance of subcellular localization of AMPK. This research will only be innovative if the ideas described here will ultimately become reality; in other words, if we can finally extrapolate all gathered knowledge to human subjects, aiming at the cure of the patient.
Conversely, from another interesting point of view, we might be able to further extract and apply this potentially advance knowledge to other diseases, such as the Wolff-Parkinson-White syndrome, a congenital cardiac abnormality associated with abnormal glycogen accumulation. In such disease, it might be interesting to study whether the shift of AMPK towards glycogen, in contrast to T2D, would lower cardiac glycogen content. Moreover, AMPK is also emerging as a possible metabolic tumor suppressor. Although there is no evidence yet, it might be worthy to further investigate whether the development of new drugs specifically aiming at shifting AMPK away from glycogen may have a potential beneficial role in cancer prevention and/or treatment.

References