Thermogenic modulation of adipose depots: A perspective on possible therapeutic intervention with early cardiorenal complications of metabolic impairment*.


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Non-standard abbreviations:
BAT, Brown adipose tissue
CA, Carbonic Anhydrase
cCRP, Cyclocreatine phosphate
HIF-1α, Hypoxia inducible factor-1α
MTDL, Multi-target Directed Ligand
NF-κB, Nuclear factor-κB
PPARγ, Peroxisome Proliferator-Activated Receptor
PRAT, Perirenal adipose tissue
PROTAC, Proteolysis activating chimeric compounds
PVAT, Perivascular adipose tissue
UCP1, Uncoupling protein 1
Abstract:
Cardiovascular complications of diabetes and obesity remain a major cause for morbidity and mortality worldwide. Despite significant advances in the pharmacotherapy of metabolic disease, the available approaches do not prevent or slow the progression of complications. Moreover, a majority of patients present with significant vascular involvement at early stages of dysfunction prior to overt metabolic changes. The lack of disease-modifying therapies affects millions of patients globally, causing a massive economic burden due to these complications. Significantly, adipose tissue inflammation was implicated in the pathogenesis of metabolic syndrome, diabetes, and obesity. Specifically, perivascular (PVAT) and perirenal adipose tissue (PRAT) depots influence cardiovascular and renal structure and function. Accumulating evidence implicates localized PVAT/PRAT inflammation as the earliest response to metabolic impairment leading to cardiorenal dysfunction. Increased mitochondrial uncoupling protein-1 (UCP1) expression and function leads to PVAT/PRAT hypoxia, inflammation, as well as vascular, cardiac, and renal dysfunction. As UCP1 function remains an undruggable target so far, modulation of the augmented UCP1-mediated PVAT/PRAT thermogenesis constitutes a lucrative target for drug development to mitigate early cardiorenal involvement. This can be achieved either by subtle targeted reduction in UCP-1 expression using innovative proteolysis activating chimeric molecules (PROTACs) or by supplementation with cyclocreatine phosphate, which augments the mitochondrial futile creatine cycling and thus, decreases UCP1 activity, enhances the efficiency of oxygen use and reduces hypoxia. Once developed, these molecules will be first-in-class therapeutic tools to directly interfere with and reverse the earliest pathology underlying cardiac, vascular, and renal dysfunction accompanying the early metabolic deterioration.

Significance statement:
Adipose tissue dysfunction plays a major role in the pathogenesis of metabolic diseases and their complications. While mitochondrial alterations are common in metabolic impairment, it was only recently shown that the early stages of metabolic challenge involve inflammatory changes in select adipose depots associated with increased uncoupling protein 1 thermogenesis and hypoxia. Manipulating this mode of thermogenesis can help mitigate the early inflammation and the consequent cardiorenal complications.
1. Introduction:

Metabolic disorders are typically associated with increased cardiovascular risk as they tend to coincide with several inflammation-driven cardiorenal and thrombotic manifestations in what became recognized as the metabolic syndrome (Castro et al., 2003; Chait and den Hartigh, 2020). Such elevated risk culminates in multiple adverse outcomes including coronary heart disease, stroke, and heart failure (Ash-Bernal and Peterson, 2006; Powell-Wiley et al., 2021; von Bibra et al., 2016). The past two decades have witnessed a tremendous increase in the prevalence of metabolic syndrome and its cardiovascular consequences (Li et al., 2021; Moore et al., 2017; Ng et al., 2021). In particular, patients suffering from diet-induced obesity (WHO, 2019) and type 2 diabetes (International_Diabetes_Federation, 2019), both considered end-stage outcomes of metabolic syndrome, together with their concurrent cardiorenal complications, have significantly increased in numbers. Such an exponential rise was possibly triggered by contemporary shifts in dietary habits associated with overeating and increased representation of refined, calorie-dense food groups that are enriched in saturated fat and simple sugars (Barbosa et al., 2022; Lutsey et al., 2008; Misra et al., 2010). The staggering cost of these health risks is an economic challenge to be reckoned with, making the search for disease modifying interventions that tackle early stages of metabolic dysfunction a priority. Nevertheless, the quest for such radical therapies to pre-empt cardiorenal complications of metabolic syndrome is hurdled by multiple obstacles. First and foremost, early stages of metabolic impairment that carry considerable cardiovascular risk do not usually meet the common diagnostic thresholds of bona fide metabolic disorders (Grundy, 2012). Additionally, several manifestations of early vascular impairment, including dysfunctional microvascular dilation and impaired blood flow auto-regulation that have serious cardiovascular implications, can occur without detectable signs of atherosclerosis or other vascular anomalies in the context of early metabolic syndrome (Tune et al., 2017).

As such, this knowledge gap of the mechanism of early pathology and the lack of therapeutic agents specifically developed to reverse this detrimental process creates a significant unmet clinical need. Recent estimates from the International Diabetes Federation put the number of diabetic patients in the Middle East and North Africa region only around 50 million, yet the number of undiagnosed and “at high risk” individuals soars to at least an additional 50 million (El-Kebbi et al., 2021; International_Diabetes_Federation, 2019). Moreover, research has shown that cardiovascular complications among patients with metabolic dysfunction are highly prevalent in our region (Bennet et al., 2013; Taleb et al., 2008). Therefore, there exists a pressing need for development of therapeutic tools tailored to reverse the cardiovascular pathology in its early stages.
2. **The mechanism of cardiorenal involvement in early metabolic impairment:**

Accumulating evidence depicts a clear association between prolonged exposure to high-caloric intake and adipose tissue inflammation with the pathogenesis of several metabolic disorders including obesity and type 2 diabetes (Choe et al., 2016). Indeed, inflammatory changes in the adipose tissue are expected to play a crucial role in the development of these conditions (Kohlgruber and Lynch, 2015; Richardson et al., 2013; Shimizu et al., 2013), especially that insulin resistance has long been recognized as an integral component of the metabolic syndrome (Reaven, 1988). Under such circumstances, the triggered inflammatory process involves several types of immune cells and cytokines. In this regard, localized alteration of the normal adipokine/cytokine profile confined to the perivascular adipose tissue (PVAT) were implicated in vascular impairment (Greenstein et al., 2009; Yudkin et al., 2005), offering a contextual framework for the occurrence of early microvascular dysfunction in absence and prior to development of hyperglycemia. Indeed, we have recently examined these changes in the context of early cardiovascular and renal complications (Al-Assi et al., 2018; Elkhatib et al., 2019; Hammoud et al., 2021). Our results indicate that localized perivascular and perirenal adipose tissue (PRAT) inflammation occurring in isolation of systemic inflammatory involvement triggered renal, vascular, and cardiac autonomic dysfunction in the early prediabetic stage. Other groups used alternative experimental approaches to reach the same conclusion that isolated PVAT inflammation is the major trigger of vascular dysfunction in metabolic impairment independent of blood glucose level, glucose tolerance, blood lipids, and body weight (Horimatsu et al., 2018).

Specifically, hyperinsulinemia associated with metabolic challenge is thought to drive adipose inflammation (Pedersen et al., 2015) by triggering adipocyte hypertrophy to accommodate increased calorie intake, leading to reduction of interstitial oxygen levels (Lee et al., 2014). Adipose tissue hypoxia triggers hypoxia inducible factor-1α (HIF-1α) and nuclear factor-κB (NF-κB) mediated transcription, leading to increased production of inflammatory mediators (Chan et al., 2016; Cole et al., 2013). Interestingly, our recent results demonstrated that PVAT/PRAT are more sensitive to these changes compared to larger adipose tissue pools previously implicated in the inflammatory and cardiovascular outcomes associated with obesity, especially visceral adipose (Beasley et al., 2009), and hence in the absence of a detectable change in systemic inflammatory cytokine levels (Elkhatib et al., 2019). This is likely to occur as a result of their unique nature, with expression of characteristic proteins including uncoupling protein 1 (UCP1) (Hildebrand et al., 2018; Szasz and Webb, 2012). UCP1 is responsible for dissipating mitochondrial energy as heat in brown adipose tissue (BAT) during non-shivering thermogenesis, and its activity and expression are associated
with increased oxygen consumption (Schneider et al., 2016; Shabalina et al., 2013; Vijgen et al., 2013). Whereas considerable evidence in the literature showed upregulation of UCP1 expression in BAT in response to increased caloric intake (Fromme and Klingenspor, 2011), our studies were the first to report this to occur in PVAT/PRAT and in a manner corresponding to changes in HIF1-α expression and concomitant with the incidence and amelioration of inflammation and renal/vascular dysfunction (Elkhatib et al., 2019; Hammoud et al., 2021). As such, the early involvement of PVAT/PRAT inflammation could be due to an augmented sensitivity to hypoxia driven by the increased UCP1 expression, which does not occur in white visceral adipose pools. A vast expanse of evidence characterized the pathways linking hypoxia to adipose inflammation involving HIF1-α and NF-κB signaling (Chan et al., 2016; Cole et al., 2013). Yet, an effort to develop therapeutic tools directly and selectively suppressing these targets has not been forthcoming and UCP1 expression/activity remain largely undruggable. Such therapy would allow intervention with early stages of metabolic impairment to reverse vascular and cardiac dysfunction in metabolic disease.

3. **UCP1-mediated thermogenesis as a therapeutic target:**

The traditional view of exploiting UCP1 as a target for the amelioration of metabolic disorders involves the opposite approach of inducing its expression in white adipose tissue (WAT) in the process termed browning (Lizcano, 2019). In this regard, it has been suggested that higher UCP1 activity and the ensuing increase in energy dissipation could improve glucose and lipid oxidation and their uptake from the circulation, which could lead to improved insulin sensitivity and reduced dyslipidemia (Kim and Plutzky, 2016). Increased UCP1 activity can be triggered by a variety of stimuli (e.g. cooling or sympathetic stimulation), and thus many agents were investigated to induce this effect in humans, including β3-adrenergic receptor agonists, capsinoids, and glucocorticoids (Cypess et al., 2015; Ramage et al., 2016; Yoneshiro et al., 2013). However, their applications are currently limited due to marginal weight loss effects or undesirable side effects. Additionally, some data suggest that overactive BAT and WAT browning may be associated with excessive release of lipids, which could contribute to atherosclerosis, hepatic steatosis and immune suppression (Tamucci et al., 2018). Indeed, early studies of transgenic mice lacking UCP1 expression showed a paradoxical resistance to diet-induced obesity, decreased adiposity, reduction of plasma lipids, and increased energetic dependence on fat oxidation (Liu et al., 2003). At the time, the investigators argued for a possible upregulation of an alternative non-shivering thermogenic mechanism to underlie these effects. Coincidentally, one of the recent views of non-pharmacological management of cardiometabolic dysfunction argues multiple organ sympathetic denervation as an approach to mitigate complications with a potential impact in reducing UCP1 expression (Kiuchi et al., 2023). Moreover, adipose browning in cancer-associated cachexia was proposed to exacerbate metabolic dysfunction.
PETRUZZELLI AND WAGNER, 2016). AS SUCH, IT IS NECESSARY TO VIEW THE IMPACT OF UCP1 EXPRESSION/ACTIVITY WITHIN ITS PARTICULAR CONTEXT AND REASONABLE TO CONSIDER ITS MIXED NEGATIVE AND POSITIVE EFFECTS (ABDULLAHI AND JESCHKE, 2016).

4. UCP1-INDEPENDENT THERMOGENESIS:

IN THE CONTEXT OF THERMOGENESIS, A GROWING BODY OF EVIDENCE HAS IMPLICATED MANY PATHWAYS OTHER THAN UCP-MEDIATED PROTON INFLUX IN MITOCHONDRIAL HEAT GENERATION. THESE INCLUDE FUTILE CREATINE CYCLING, CALCIUM CYCLING, LIPOSYCLE/RE-ESTRIFICATION CYCLING, AND ADP/ATP CARRIER PROTON LEAK. IMPORTANTLY, RECENT STUDIES HIGHLIGHTED MITOCHONDRIAL FUTILE CREATINE CYCLING AS AN ADDITIONAL ENERGY DISSIPATION MECHANISM SPECIFICALLY ACTIVATED IN ADIPOSE TISSUE IN RESPONSE TO INCREASED CALORIC INTAKE (KAZAK ET AL., 2019). IN THIS FRAMEWORK, CONTINUOUS ALTERNATING CREATIVE PHOSPHORYLATION AND DEPHOSPHORYLATION PROCEED TO DISSIPATE ENERGY AS HEAT IN ADIPOCYTES (KAZAK ET AL., 2015). THIS PROCESS IS TRIGGERED BY A COORDINATED ACTIVATION OF α1- AND β3 ADRENERGIC RECEPTORS INDUCED BY THERMOGENIC STIMULATION, FOLLOWED BY Gαs AND Gαq SIGNALING, RESPECTIVELY (KAZAK ET AL., 2022; RAHBANI ET AL., 2021; SUN ET AL., 2021). MITOCHONDRIAL PATCH CLAMP EXPERIMENTS REVEALED THE OCCURRENCE OF FUTILE CREATINE CYCLING IN UCP1-POSITIVE AND UCP1-NEGATIVE BEIGE ADIPOCYTES (BERTHOLET ET AL., 2017). IN CONTRACT TO OBSERVATIONS IN UCP-1 KNOCKOUT MICE, ADIPOCYTE-SPECIFIC DELETION OF THE ENZYMES INVOLVED IN FUTILE CREATINE CYCLING DAMPENS ENERGY EXPENDITURE AND Augments sensitivity to diet-induced obesity. Additionally and with direct relevance to the early induction of cardiorenal complications, recent evidence implicated impaired phosphocreatine metabolism in the development of white AT inflammation (MAQDASY ET AL., 2022). Not only do the available data suggest a reciprocal relationship between creatine cycling and UCP1 activities, evidence also points out that creatine cycling is associated with less oxygen consumption than that resulting from UCP1 activity (KAZAK ET AL., 2015; KAZAK ET AL., 2019). Cellular creatine content is either endogenously biosynthesized or imported from the extracellular medium, both processes limited by maximal activities of the corresponding enzyme/transporter. Nevertheless, an acute increase in cold-induced thermogenic activation following creatine supplementation to young, healthy, and lean vegetarian adults, who have low circulating creatine levels, was not observed (CONNELL ET AL., 2021). IN THIS REGARD, ANIMAL EXPERIMENTS DEMONSTRATED THE NECESSITY FOR A certain metabolic imbalance to uncover the role of creatine cycling-dependent thermogenesis, an observation pertaining directly to targeting this pathway in metabolic disease.

and it was shown that the inhibition of SERCA2b impairs UCP1-independent beige adipocyte thermogenesis in mice and in humans (Ikeda et al., 2017). Alternatively, lipolysis/re-esterification of triglycerides constitutes an energetically futile cycle that drives UCP1-independent consumption of cellular ATP based on the energy demand of triacylglycerol breakdown and acylglycerol synthesis (Oeckl et al., 2022). Cold exposure, adrenergic stimulation, and PPARγ agonism collectively increase lipolysis and fatty acid synthesis (Guan et al., 2002; Mottillo et al., 2014; Sepa-Kishi et al., 2019; Yu et al., 2002). Interestingly, this pathway of heat production is triggered by cold exposure not only BAT, but also in WAT (Brooks et al., 1982). Finally, adenine nucleotide translocase, which exchanges mitochondrial ATP for cytosolic ADP, induces proton cycling at high membrane potential in a UCP1-independent manner (Bertholet et al., 2019; Brown et al., 2010). This type of proton leak requires free fatty acids, and is suppressed by the exchange activity of the carrier, indicating that proton leak is regulated by the cellular ATP abundance (Bertholet et al., 2019; Brand et al., 2005; Shabalina et al., 2006). Adenine nucleotide translocase isoform expression increases in response to insulin and PPARγ agonism to support oxidative metabolism, and occurs at higher levels in sub-cutaneous rather than visceral adipose tissue (Gavaldà-Navarro et al., 2015).

5. Approaches modulating thermogenesis and other mitochondrial targets as potential therapeutic interventions for early cardiorenal complications:

Indeed, mitochondrial alterations in the context of metabolic dysfunction is a widely accepted concept. Evidence suggests that many of the proteins required for normal mitochondrial function including the respiratory chain complexes were downregulated in animal models of prediabetes and diabetes with cardiac dysfunction without overt macrovascular involvement (Szücs et al., 2019; Wang et al., 2020). Moreover, data from diabetic mouse models showed that the reduced ATP production under these circumstances is associated with mitochondrial depolarization (Veeranki et al., 2016). Nevertheless, multiple studies in animal models and humans showed that these changes are associated with increased oxygen consumption (Federico et al., 2021) similar to the observation of increased hypoxia in the PVAT/PRAT in prediabetes (Dwaib et al., 2021; Elkhatib et al., 2019; Hammoud et al., 2021). Additionally, mitochondrial carbonic anhydrases (CAs) are also among the factors contributing to cellular damage in diabetes and metabolic dysfunction (Shah et al., 2013b). Interestingly, high-glucose exposure triggered an increase in mitochondrial oxygen consumption rates and augmentation of the production of reactive oxygen species that were ameliorated by the inhibition of mitochondrial CAs (Shah et al., 2013a). Moreover, mitochondrial CA inhibitors have been recently proposed as anti-obesity drug candidates (Supuran, 2022). These findings among others have triggered an interest in developing mitochondria-based therapies for metabolic syndrome (AlZaim et al., 2022; Bhatti et al., 2017).
As such, the development and screening of novel molecules that selectively affect adipose tissue inflammatory pathways through interference with thermogenesis represent a prudent target of research in the immediate future. Thus far, a direct therapy geared to reverse cardiovascular complications of metabolic dysfunction has not been forthcoming. Although adipose inflammation had been implicated in this process, no specific agents were developed to ameliorate this pathology. To the best of our knowledge, the currently available UCP1 inhibitors possess fairly high IC₅₀ values (~20 μM) (Rial et al., 2011) precluding systemic administration, effectively rendering UCP1 an undruggable target using the present tools. To circumvent this, we propose using proteolysis activating chimeric (PROTAC) technology to develop drug leads selectively targeting UCP1. A PROTAC is a heterofunctional small molecule combining a protein ligand (e.g. a channel or enzyme inhibitor) and another molecule capable of recruiting an E3 ubiquitin ligase causing target protein ubiquitination and downregulation by proteasome degradation (Sakamoto et al., 2001). Hence, instead of acting as a conventional enzyme inhibitor or channel blocker stoichiometrically in a traditional “occupancy-based” pharmacology, PROTACs are able to produce sub-stoichiometric effects where one molecule can lead to the ubiquitination and degradation of several target molecules in what came to be described as “event-driven” pharmacology (Lai and Crews, 2017). On the other hand, cyclocreatin phosphate (cCrP) is a membrane permeable creatine analogue (Kurosawa et al., 2012) supported by robust evidence to preserve tissue ATP levels and reverse inflammatory and apoptotic damage triggered by ischemia (Elgebaly et al., 2019). Specifically, cCrP treatment was shown to attenuate the earliest consequences of ischemia in cardiac tissues including inflammatory cytokine production, immune cell recruitment, and extra-cellular matrix remodeling (Elgebaly et al., 2019). Moreover, in the context of adipose tissue remodeling in early metabolic dysfunction, recent research showed that futile creatine cycling is an alternative mitochondrial response selectively activated by increased caloric intake, in parallel to UCP1 upregulation, to dissipate excess energy (Kazak et al., 2019). Enhancement of creatine cycling reduces UCP1 activity. The proposed model for action of these tools is depicted in Figure 1.

On the other hand, several drug lead generation programs addressed the need for drugs targeting low-grade adipose inflammation using innovative chemistry to render efficacious molecular tools. The use of click chemistry enabled the design of simple synthetic schemes generating multi-target directed ligands (MTDLs) via the fusion of disparate pharmacophores into the same molecular scaffold in a manner that might not have been achieved as easily otherwise (Alaaeddine et al., 2021; Elzahhar et al., 2019; Elzahhar et al., 2021; Moussa et al., 2018). This approach has proven useful in developing compounds with superior activity against acute and chronic inflammatory models, macrophage differentiation and pro-inflammatory polarization, neuroinflammation, and adipose inflammation. In this regard, one
of the leading molecular candidates demonstrated cyclooxygenase-2 (COX-2) and 15-lipoxygenase (15-LOX) inhibition potencies that are slightly less than reference inhibitors, combined with a partial Peroxisome Proliferator-Activated Receptor-γ (PPARγ) agonistic activity, making it ideally suited to produce a synergistic effect in reducing adipose tissue inflammation while decreasing the likelihood of adverse effects resulting from excessive inhibition or stimulation of a given pathway (Elzahhar et al., 2019). Importantly, following this methodology, our group designed and tested anticancer MTDL that inhibited tumor-associated CA isoforms, in addition to typical pro-inflammatory targets such as cyclooxygenase and lipoxygenase, which proved to harbor superior cytotoxic activity against cancer cells (Elzahhar et al., 2020). Following the same rationale, we envision that developing MTDLs combining mitochondrial CA inhibitory activity with the anti-inflammatory impact of COX-2 and 15-LOX inhibition might prove useful in combating PVAT/PRAT inflammation triggered by increased UCP1-mediated thermogenesis and hypoxia. While there are no data to describe a direct interaction between mitochondrial CAs activity and UCP1-mediated thermogenesis, the available evidence indicates that mitochondrial CA inhibition does not only reduce mitochondrial depolarization (Micheli et al., 2022) expected to increase with augmented UCP1 activity, but is also associated with decreased HIF1-α (Bernardino et al., 2019), which is in line with the reduced oxygen consumption reported previously to occur with CA inhibition (Shah et al., 2013a). As such, our proposed rationale for these molecular candidates would be best represented by the pharmacophore fusion scheme depicted in Figure 2. A similar view can be adopted to incorporate CA inhibitory activities and PPARγ stimulatory activity within one scaffold intended to target adipose inflammation in the future. The same approach in chemical synthesis enhances the feasibility of the generation of the anti-UCP1 PROTAC molecule, which is expected to have a complex structure. The envisioned click chemistry scheme allows the development of a rather comprehensive library of derivatives with a broad range of drug-likeness characteristics with potentially permissive pharmacokinetic profiles.

6. Novel pharmacological tools: Pros and cons

We believe that the design and evaluation of the effect of such tools on the cardiorenal and vascular dysfunction associated with the early stages of metabolic deterioration might pose a significant step towards the development of disease-modifying therapies. Such an endeavor requires the development of relevant disease models. In the past decade, we developed and characterized a rat model of early metabolic dysfunction showing isolated PVAT/PRAT inflammation and cardiovascular dysfunction that would serve as an appropriate test subject for this purpose (Elkhatib et al., 2019). Rats chronically exposed to a mild increase in saturated dietary fat and refined sugars demonstrate isolated PVAT inflammation and cardiovascular dysfunction in absence of hyperglycemia, obesity, or systemic
inflammation. These rats demonstrate an altered body composition with an increased fat:lean ratio and a reduced metabolic efficiency consistent with the increased mitochondrial UCP1 expression (Dwaib et al., 2021). Nevertheless, a thorough assessment of the positive and negative impact of the proposed drug classes would be required in this model, in addition to healthy animals and in animal models of other metabolic disease, particularly these with renal and hepatic complications. This is particularly necessary due to the lack of data on the prolonged and generalized interference with mitochondrial thermogenic pathways. Yet, the relative safety, and effects in terms of cold intolerance and metabolic efficiency, for the downregulation of UCP1 expression and function could be inferred from studies on transgenic mice lacking UCP1 (Liu et al., 2003), or these using inorganic phosphate supplementation as a potential inhibitor of UCP1 (Dwaib et al., 2021), where no detrimental effects were observed on gross, functional, or metabolic activities or blood chemistry parameters. However, the long-term adaptive effects of these tools might not be easy to predict and harmful outcomes are not to be precluded without adequate investigation, since both UCP1-mediated thermogenesis and futile creatinine cycling were implicated in intricate interactions among not only the adipose tissue depots and the autonomic nervous system, but also with several components of the immune system (Jun et al., 2018; Rahman and Jun, 2022). Additionally, long-term alteration of thermogenesis might yield a yet to be defined effect on the bioenergetic balance of carbohydrate vs. lipid oxidation as a fuel source with potential consequences on hepatic metabolic pathways. On the other hand, both phosphocreatine and CA inhibitors might have additional effects that are not related to lipid metabolism. For instance, long-term phosphocreatine supplementation will be expected to have an impact on skeletal muscle energetics, while chronic CA inhibition might interfere with the processes regulating the acid-base balance in the body, despite the possible isoform selectivity of the proposed candidate molecules.

7. Conclusions:

All in all, it is our view that increasing the delivery of cCrP to PVAT/PRAT, controlled downregulation of UCP-1, and/or modulation of mitochondrial CA activity will ameliorate the hypoxic state observed in this fat depot by possible modulation of UCP1-dependent thermogenesis. Treatment will potentially decrease the adipocyte reliance on UCP1 activity, and hence improve the efficiency of oxygen consumption. Mitigation of PVAT/PRAT hypoxia will ameliorate the consequent inflammatory changes and reduce the paracrine spillover into vascular, cardiac, and renal tissues. This is expected to interrupt the pathological process leading to cardiovascular dysfunction in early metabolic deterioration, and thus avert the root cause of these complications. As such, this highlights the importance of the proposed molecules as innovative first-in-class therapeutic tools with properties allowing for tailored intervention in the earliest
detectable pathology preventing future vascular deterioration as the patient’s metabolic status degenerates into diabetes.

**Data Availability Statement:** This article contains no datasets generated or analyzed during the current study.

**Author contributions:**

*Wrote or contributed to the writing of the manuscript:* El-Yazbi AF, Elrewiny MA, Habib HM, Eid AH, Elzahhar PA, and Belal ASF.
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Footnotes:

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Figure Captions:

Figure 1. The pathological model for development of early localized adipose tissue inflammation and the proposed mechanism of action of drugs modulating this effect. Increased caloric intake from saturated fat and refined sugars triggers hyperinsulinemia, leading to adipocyte hypertrophy, which, together with increased uncoupling protein 1 (UCP1) expression, makes these select adipose tissue depots more vulnerable to hypoxia and inflammation with the ensuing cardiorenal dysfunction. Modulation of UCP1 activity, either via activating futile creatine cycling bypassing UCP1-mediated thermogenesis or through subtle downregulation/normalization of UCP1 expression, constitutes a potential strategy for drug development to selectively target localized adipose tissue inflammation and the consequent cardiorenal complications. Molecular and cellular processes perceived to be detrimental are outlined in red, while the envisioned corrective actions of the proposed therapeutic tools are outlined in green. cCRP, Cyclocreatine phosphate; HIF-1α, Hypoxia inducible factor-1α; NF-κB, Nuclear factor κB; PRAT, Perivascular adipose tissue; PROTAC, Proteolysis activating chimeric compound; PVAT, Perivascular adipose tissue; UCP1, Uncoupling protein 1.

Figure 2. The proposed pharmacophore fusion scheme for the multi-target directed ligands inhibiting carbonic anhydrase together with COX-2 and 15-LOX. Building on our 1,2,3-triazole anti-inflammatory lead compound (A) and the previously reported 1,2,3-triazole sulfonamide inhibitor of human carbonic anhydrase (hCA) II, VA and VB isoforms (B), the mitochondrial hCA VA and VB inhibitory activities of the proposed sulfonamido triazoles (C and D) whose hCA II inhibiton is already established among other CA isoforms might prove useful in combating PVAT/PRAT inflammation resulting from increased UCP1-mediated thermogenesis, increased oxygen consumption and hypoxia.
Mild hyper caloric intake (higher saturated fat and simple sugars)

Hyperinsulinemia

- Adipocyte hypertrophy
  - ↑ tissue hypoxia, ↑ HIF1-α expression
  - Monocyte recruitment, macrophage activation, ↑ NF-κB signaling
  - ↑ inflammatory cytokine production
  - Vascular, cardio autonomic, and renal dysfunction

- Increased UCP1 expression specifically in PVAT/PRAT
  - ↑ creatine cycling, bypass UCP1, ↓ O₂ consumption
  - Controlled reduction of UCP1 expression, ↓ O₂ consumption
  - ↓ manifestations of adipose inflammation, correct early cardiovascular and renal dysfunction

- cCrP supplementation
- UCP1-targeting PROTACs

Figure 1
Figure 2

(A) COX-2 and 15-LOX inhibitor

Our previous work


(B) COX-2 & 15-LOX inhibitor

\[ K_i \text{ hCA II} = 7.7 \text{ nM} \]
\[ K_i \text{ hCA VA} = 9.3 \text{ nM} \]
\[ K_i \text{ hCA VB} = 11.4 \text{ nM} \]


(C) Unpublished work

2-digit Nanomolar inhibitors of hCA II, IV & IX

(D) Our previous work