Review

The 2012 CDA-CIHR INMD Young Investigator Award Lecture: Dysfunction of Adipose Tissues and the Mechanisms of Ectopic Fat Deposition in Type 2 Diabetes

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Abstract

Ectopic fat deposition in skeletal muscles, liver, heart, and other tissues has been closely linked with the development of lean tissues’ insulin resistance and progression toward type 2 diabetes mellitus. Mechanisms of overexposure of these tissues to fatty acids include increased de novo lipogenesis, impaired fatty acid oxidation and increased fatty acid flux to these organs. White adipose tissues are the main organs responsible for the regulation of circulating fatty acids. It has been clearly demonstrated that pre-diabetes individuals and individuals with diabetes display impaired adipose tissue dietary fatty acid storage that may lead to increased circulating flux and exaggerated lean tissue fatty acid exposure. Additionally, brown adipose tissue depots are less metabolically active in individuals with type 2 diabetes. We have developed a series of novel in vivo investigative tools using positron emission tomography to comprehensively assess postprandial interorgan fatty acid partitioning and white and brown adipose tissue metabolism in subjects with pre-diabetes and type 2 diabetes. Our findings shed new lights into the sophisticated mechanisms that regulate fatty acid partitioning and energy homeostasis during the development of type 2 diabetes. New links between abnormal dietary fatty acid metabolism and early myocardial metabolic and functional defects are now being uncovered in humans with the hope to find novel ways to predict and avoid the devastating complications of diabetes.

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Résumé


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Introduction: A Tribute to My Mentors and Collaborators

Malcolm Gladwell captured in his best-seller book “Outliers” what I believe should be in the mind of anyone receiving a biomedical research award nowadays: “Success is grounded in a web of advantages and inheritances, some deserved, some not, some earned, some just plain lucky.” I am a prime example of a clinician-scientist who is the product of a perfectly favourable environment for the development of a biomedical research career. Throughout my training and career, I benefited from my interactions with a series of individuals who shaped my professional life. They created the environment that was conducive for the methodological innovations and original conceptual insights that led to my nomination to the CDA-CIHR INMD Young Investigator Award.

Two individuals stand out as the most important of them all, as they not only gave impulse and direction to my research career, but also profoundly transformed my values and vision and made me a true team player and collaborator. The first of these individuals is Dr. Diego Bellabarba who was the Director of the Endocrinology Training Program at the Université de Sherbrooke when I was a medical clerk and, later, an endocrinology resident. Diego not only transmitted his passion for medicine, endocrinology and research, he also introduced me to the humanistic views and culture that are now the motive force of my career and, in many ways, of my personal life. Diego was truly a father figure to me. The second mentor is Dr. Gary Lewis at the University of Toronto, my postdoctoral fellowship supervisor from 1997 to 2001. Gary communicated his passion for research on diabetes and lipid metabolism and truly defined the conceptual path at the basis of a large part of my independent career thus far (1). At the time of my training with him, Gary was one of the few integrative physiologists active in metabolic research in humans, a discipline that is now in high demand. Under his guidance, I learned all of the basic skills in metabolic research that allowed my laboratory to develop the novel methodological tools that will be overviewed in the present article.

Other current collaborators also deserve a special mention as they were instrumental in the development of some of the conceptual and/or methodological advances that will be underlined herein: Dr. Eric E. Turcotte, Head of the Positron Emission Tomography (PET) Investigation Unit, Dr. Roger Lecomte, Head of the Preclinical PET Investigation Unit and Dre. Brigitte Guérin, Head of the Radiochemistry Unit at the Centre de recherche clinique Étienne-Le Bel (CRCEL), Dr. Denis Richard of the Obesity Research Chair at Université Laval and Dr. François Haman of the University of Ottawa. None of the work my laboratory has accomplished would have been possible without the outstanding contributions of these good friends.

The concept of disordered storage of fatty acid in adipose tissues in the development of type 2 diabetes

Ample evidence from preclinical investigations as well as experimental studies in humans supports the notion that excess exposure of lean tissues to fatty acids is a key factor leading to insulin resistance (IR) and impaired glucose-stimulated insulin secretion (GSIS), the 2 pathophysiological hallmarks of type 2 diabetes mellitus (see [1–3] for reviews). This process is generally referred to as “lipotoxicity” (4). The underlying mechanisms by which excess lean tissue fatty acid exposure occurs and the role of lipotoxicity in the natural history of type 2 diabetes in humans have been the focus of our laboratory since its inception in 2001. The most recognized in vivo marker of lean tissue fatty acid overexposure and lipotoxicity is ectopic fat deposition (i.e. excess intracellular deposition of triglycerides [TG]) (3,5).

Excess exposure of lean tissues to fatty acids may occur via 3 categories of potentially overlapping mechanisms (2). The first category of mechanisms relates to defective lean tissue fatty acid metabolism, including 1) impaired NEFA oxidation (OxNEFA) that likely contributes to ectopic fat deposition in skeletal muscles (see [2] for review), and 2) increased synthesis of fatty acids from other carbon substrates (de novo lipogenesis [DNL]) that is likely the main contributor of liver ectopic fat deposition (see also [2] for review). The second mechanisms are those related to adipose tissue metabolic dysfunction and/or impaired expansion (see [1–3] for reviews), namely 1) excess intracellular lipolysis of prestored TG, the major contributor of plasma nonesterified fatty acid appearance during fasting (RaNEFA), and 2) impaired adipose tissue storage of fatty acids produced by lipolysis of TG-rich lipoproteins (mainly chylomicrons) (6–10), a process referred to as “NEFA spillover” and that also contributes to RaNEFA in the postprandial and postabsorptive states. Using stable isotopic tracer techniques, we found clear evidence for increased NEFA spillover in subjects with pre-diabetes and diabetes (9,10). Others have demonstrated using arterio-venous gradient techniques and/or isotopic tracer methods with biopsies that NEFA spillover originates in the adipose tissue microcirculation from impaired adipose tissue fatty acid storage (see [2] for an extensive and critical review). The third category, excess lean tissue uptake of dietary fat (Uptakedietary fat), may relate to impaired adipose tissue postprandial storage of dietary fatty acids, increased intestinal fat absorption and/or directly increased fatty acid uptake in lean tissues. These latter mechanisms have been thus far poorly characterized in humans because of methodological limitations. This challenge has been the focus of novel methodological developments in our laboratory.

Our contribution to advances in the quantification of organ-specific fatty acid partitioning

PET is a nuclear medicine approach that allows the quantification of 2 coincident gamma rays emitted from a positron-emitting label contained in a tracer tailored to quantify a biological process such as uptake of a metabolite or binding to a receptor. For example, 14-R,S-18F-6-thia-heptadecanoic acid (18FTHA), a long-chain fatty acid analog that accumulates in oxidative and nonoxidative cellular pathways (11), offers the possibility to measure integrated tissue uptake of long-chain fatty acids in vivo in humans with PET. Three PET acquisition modalities are useful as part of our metabolic investigations: 1) static (or volumetric) acquisition, in which all coincident radioactive events within a given space (voxel) are summed up over time to give a single 3-dimensional image to determine whole body tracer biodistribution; 2) dynamic acquisition, in which each voxel is associated to a time of detection, allowing the reconstruction of 4-dimensional images to study time-dependent processes such as tracer uptake rate and oxidative and nonoxidative metabolic rates; and 3) electrocardiomyographic (ECG)-gated dynamic acquisition, in which PET dynamic acquisition of the heart is synchronized to the cardiac cycle, allowing the determination of cardiac volumes to assess ventricular function.

Using PET technology developed by the team of Lecomte et al. (12) and Marriot et al. (13) that is adapted to small animals (μPET), we investigated fatty acid metabolism in the heart of a series of animal models, including a nutritional rat model of type 2 diabetes that we developed using high fructose/high fat feeding combined with administration of a small dose of streptozotocin (the HFHFS rat) (14). Like most patients with type 2 diabetes, these animals are characterized by insulin resistance, hypoadiponectinemia, mild hyperglycemia and hypertriglyceridemia and adipocyte hypertrophy (14,15). However, this model is not characterized by significant degree of obesity, allowing the assessment of the effect of a type 2 diabetes like condition without the confounding effect of obesity. Using sequential dynamic ECG-gated μPET scanning after the intravenous (i.v.) administration of 11C-acetate, 18FDG...
and $^{18}F$THA, we measured myocardial blood flow, oxidative metabolism, glucose and NEFA use and left ventricular function. We found that HFHFS rats have reduced left ventricular contractility with reduced stroke volume associated with preserved oxidative metabolism and blood flow (14). This reduced ratio of myocardial mechanical work on oxygen consumption is typical of diabetic cardiomyopathy (16). In euglycemic hyperinsulinemic condition, the heart of the HFHFS rat shows reduced glucose use but normal cardiac NEFA uptake (14). Cardiac NEFA uptake rate was even reduced in the fasting condition due to significant reduction in RaNEFA from adipose tissue lipolysis. This phenomenon is also typical of patients with type 2 diabetes and is likely due to impaired adipose tissue catecholamine sensitivity (see our recent review [17]). These data excluded a significant contribution of RaNEFA to cardiac lipotoxicity in this model, stimulating us to investigate the contribution of dietary fat from circulating TG-rich lipoproteins in this process because the heart can efficiently use this source of fatty acids (16,18). We found that HFHFS rats show a marked increase in $^{18}F$THA myocardial uptake when this tracer is administered by gavage (14), suggesting an exaggerated channelling of dietary fatty acids toward the myocardium in type 2 diabetes as a contributor of the cardiac metabolic abnormalities and cardiomyopathy associated with this disease. This possibility was also supported by myocardial lipoprotein lipase (LpL) overexpression experiments in mice (19) and by altered cardiac glucose uptake induced by acute hypertriglycerideremia in rats (20).

The results obtained in the HFHFS rat model attracted our attention to the role of chylomicron-derived fatty acids in ectopic fat deposition during the development of type 2 diabetes. Our previous studies in humans also suggested that fatty acid spillover (i.e. excess RaNEFA from chylomicron-TG lipolysis during the postprandial state) is not fully established in the pre-diabetes state whereas postprandial hypertriglycerideremia is present (9,10,21). We also showed that increased postprandial RaNEFA does not contribute to increased fatty acid uptake in skeletal muscles of subjects with type 2 diabetes because of reduced postprandial muscle blood flow (22). Given the limitations of available techniques to study in vivo TG-derived fatty acid uptake (see [2] for review), we setup to establish a novel, noninvasive method to determine whole body, organ-specific dietary fatty acid partitioning and uptake in humans using oral administration of $^{18}F$THA with sequential dynamic and whole body static PET acquisitions. The use of $^{18}F$THA as a tracer for this purpose was justified based on the following rationale: 1) the very high sensitivity of PET, allowing quantification of uptake in all organs, including the heart, skeletal muscles and adipose tissues, 2) $^{18}F$THA accumulation in oxidative and non oxidative cellular pathways, allowing the measurement of integrated uptake over time, even in organs that oxidize and eliminate fatty acids very rapidly, despite the relatively slow dietary fatty acid transport rate to organs, and 3) the similar tissue uptake rates of palmitate and $^{18}F$THA in vivo (23,24). We demonstrated in rats and humans that orally administered $^{18}F$THA reaches circulation in chylomicron-TG at similar rate than $^3$H-triolein, that it redistributes to other circulating lipids (NEFA, VLDL-TG, phospholipids) as dietary long-chain fatty acids, that it allows the assessment of chylomicron-TG production into the thoracic duct and uptake and partitioning of dietary fatty acids in most tissues in humans (25). An important inherent limitation of this novel method is the incapacity to distinguish oxidative from non-oxidative dietary fatty acid metabolism. This method also assumes that hydrolysis rate of $^{18}F$THA containing chylomicron-TG by LpL is the same than native chylomicron-TG at the tissue level. It should also be mentioned that assessment of dietary fatty acid uptake over time in adipose tissues and the liver is underestimated due to significant recirculation of $^{18}F$THA as NEFA and VLDL-TG, respectively. However, the latter also occurs with endogenous long-chain dietary fatty acids and thus reflects normal physiological dietary fatty acid partitioning. Intestinal accumulation of $^{18}F$THA furthermore limits the measurement of dietary fatty acid uptake in most intra-abdominal organs due to radioactivity spillover. Finally, the need for sequential computed tomography (CT) acquisitions limits the radioactivity dose of each of these scans that can be safely administered using the currently available PET/CT scanners at our institution, thus reducing the CT image quality and our capacity to perform volumetric assessments of most tissues.

**Organ-specific dietary fatty acid partitioning in pre-diabetes**

We recently applied our novel technique to determine organ-specific dietary fatty acid partitioning in subjects with pre-diabetes (impaired glucose tolerance) (26) (Fig. 1). As expected from finding of other groups using stable or radioactive fatty acid

![Figure 1. Organ-specific partitioning of dietary fatty acids. Anterior-posterior whole body PET acquisition performed 6 hours after oral administration of 14(R,S)-$^{18}F$-FTHA. $^{18}F$ activity is clearly visible in the thoracic duct outlet (green arrow), stomach (blue arrow), liver (red arrow) and bladder (black arrow, from renal excretion of $^{18}F$THA metabolites) (Noll C, Kunach M, Labbé MS, Turcotte EE and Carpentier AC, unpublished observation).](image-url)
Brown adipose tissue metabolism in humans

Brown adipose tissue (BAT) is a specialized tissue whose function is to produce heat that is found in abundance in interscapular, subscapular, axillary, perirenal, and periaortic regions in small mammals (28). The presence of functional BAT in adult humans was only recently widely acknowledged. Indeed, PET/CT scanning investigations aimed at detecting tumoral tissue with the glucose analogue 18FDG revealed symmetrical 18FDG fat depots mostly located in the supraclavicular and paravertebral regions of the body (29). Those metabolically active fat depots were a posteriori demonstrated to have all the characteristics of BAT (30–32).

The supraclavicular depot is the most prevalent BAT in humans and the one with the highest 18F-FDG uptake activity after exposure to cold, as we showed in the largest epidemiological study on human BAT published to date (29). Higher prevalence of 18FDG BAT sites is determined by a series of factors including colder temperature preceding PET/CT scanning procedures, female sex, younger age and lower body mass index and body fat content (29,31,33). It is also reduced by the presence of diabetes and use of β-adrenergic blockers (29,33). The prevalence of spontaneously detectable BAT by 18FDG (metabolically active) range from 2% to 7% in large cohorts of patients evaluated for cancer (29,31,34). The prevalence of some metabolically active BAT using 18FDG is, however, much higher (30% to 100%) in small cohorts of acutely cold-exposed healthy subjects (32,33,35,36). The total volume of BAT 18FDG activity (a surrogate but thus far gold-standard measure of total volume of activated BAT) amounts to <50 g in most individuals, but this figure depends critically on the type and intensity of prior cold exposition and on definition criteria for positive BAT 18FDG uptake. In the absence of universally accepted definition criteria, it is important to take these considerations into account when comparing results of different groups of investigators.

In humans, there is now evidence that BAT not only can use glucose, but is also a bona fide thermogenic organ during acute cold exposure (35–37). The contribution of BAT to energy expenditure, however, is currently debated. Some groups report that, on cold exposure or capsainoid administration, whole body energy expenditure increases only in those individuals demonstrating the presence of BAT (according to any uptake of 18FDG above a defined threshold) (38–42). The inverse relationship between BAT prevalence and body mass index or % adiposity in humans also supports a possible role for BAT in the regulation of caloric balance in humans (29,31,33,43). Resting metabolism and cold-induced thermogenesis were also correlated with BAT activity in another study (44). Acute cold exposure was uncontrolled and likely not equivalent between subjects in most previously published studies. In fact, it makes little sense, from the laws of thermodynamics, that, in the face of similar cold stress, whole body energy expenditure would rise in one person and not in another. One would rather expect, as we demonstrate using a unique setup to carefully control cold exposure with a liquid conditioned suit allowing simultaneous measurement of heat dissipation, muscle shivering activity and skin and body core temperature (36) that whole body energy expenditure rises equivalently but is driven by shivering in those without BAT or by non-shivering thermogenesis in those with BAT. Indeed, we found a significant inverse relationship between BAT total volume of activity and muscle shivering activity on identical cold exposure in healthy men and men with diabetes that have thus far participated in our investigations (Fig. 2). Our findings suggest that the main function of BAT metabolic activity, at least on acute cold exposure in the fasting state, is to produce heat to protect against cold.

Is there a role for BAT in postprandial TG and energy metabolism in humans?

From animal studies, since the pioneering work of Rothwell and Stock (45), evidence that BAT adaptive thermogenesis counteracts changes in energy balance and could therefore protect against the development of obesity and type 2 diabetes has been strongly supported by some (28,45–51) but firmly contested by other investigators (52,53). Recently, BAT was shown to very efficiently metabolize TG from circulating lipoproteins in cold-exposed mice (54), consistent with the known induction of LpL expression during cold and high fat feeding-induced BAT activation (55–57) and with the role of BAT in the hypolipemic effect of PPARα treatment (58). We also demonstrated that cold-activated BAT uses circulating NEFA (36), but so far BAT role in TG clearance has not been studied in humans. In view of the known metabolic inflexibility of white adipose tissues, including resistance to both insulin-mediated storage of dietary fatty acids and catecholamine-mediated lipolysis of intracellular TG, leading to reduced flexibility of systemic fatty acid fluxes during meal intake, we recently suggested (17) that BAT may also display reduced meal and sympathetically-induced activation that may contribute to the abnormal dietary fatty acid partitioning seen in insulin resistant subjects (2,26). Despite the lack of reduction in circulating fasting plasma TG during acute cold-induced activation in our current human investigations (36), one cannot rule out a significant role of BAT in the regulation of post-prandial lipid metabolism in humans at this point.

Future Perspectives

One outstanding question is whether increased dietary fatty acid partitioning to the heart and early cardiac dysfunction in pre-

Figure 2. Inverse relationship between shivering intensity determined by electromyography and volume of active BAT determined by whole body PET/CT scanning 30 minutes after iv.18FDG in healthy subjects (n=12, open circles) and in subjects with type 2 diabetes (n=3, closed circles) analyzed thus far in our laboratory (Labbé MS, Blondin DP, Haman F, Turcotte EE, Richard D, Carpentier AC, unpublished observation). With the exclusion of one outlier healthy control, the relationship assumes an exponential decay function.
intake on dietary fatty acid partitioning and cardiac metabolism and function in subjects with impaired glucose tolerance. We are also currently testing whether cold acclimation over a 4-week period may increase BAT volume and metabolic activity in healthy individuals. If this proves to be possible, as our preliminary results suggest (Fig. 3), we will then be able to test whether increasing BAT metabolic activity may be associated with beneficial systemic metabolic effects to eventually counter weight gain and abnormal postprandial energy and lipid metabolism in pre-diabetes and diabetes. We will be able to test whether pre-diabetes and diabetes are conditions associated with both WAT and BAT metabolic dysfunctions and whether the latter may play a role in the deposition of ectopic fat and development of diabetes and its complications (Fig. 4). The outcome of these investigations will hopefully be the identification of novel target mechanisms for the monitoring of progression, prevention and treatment of type 2 diabetes and its cardiac complications.

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References


