

# Nutrition and Metabolic Correlates of Obesity and Inflammation: Clinical Considerations<sup>1–3</sup>

Amy R Johnson<sup>4</sup> and Liza Makowski<sup>4,5\*</sup>

<sup>4</sup>Department of Nutrition, Gillings School of Global Public Health, and <sup>5</sup>School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC

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## Abstract

Since 1980, the global prevalence of obesity has doubled; in the United States, it has almost tripled. Billions of people are overweight and obese; the WHO reports that >65% of the world's population die of diseases related to overweight rather than underweight. Obesity is a complex disease that can be studied from “metropolis to metabolite”—that is, beginning at the policy and the population level through epidemiology and intervention studies; to bench work including preclinical models, tissue, and cell culture studies; to biochemical assays; and to metabolomics. Metabolomics is the next research frontier because it provides a real-time snapshot of biochemical building blocks and products of cellular processes. This report comments on practical considerations when conducting metabolomics research. The pros and cons and important study design concerns are addressed to aid in increasing metabolomics research in the United States. The link between metabolism and inflammation is an understudied phenomenon that has great potential to transform our understanding of immunometabolism in obesity, diabetes, cancer, and other diseases; metabolomics promises to be an important tool in understanding the complex relations between factors contributing to such diseases. *J Nutr* 2015;145:1131S–6S.

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## Obesity and Inflammation

Over the past 30 y, the prevalence of obesity has increased epidemically in both Western nations and in developing countries, with little hope for effective treatments on the horizon (1, 2). More than one-third of adults and almost 1 in 5 children in

the United States are obese. Globally, the WHO estimates that 500 million adults and almost 43 million children <5 y are obese (3). Childhood obesity is of particular concern because health care professionals are increasingly treating children for conditions that were traditionally considered to be adult diseases such as type 2 diabetes, high blood pressure, insulin resistance, and dyslipidemia (4, 5).

Importantly, obesity is a preventable *disease*. In the United States, the American Medical Association recently classified obesity as a disease (6). There are various complications associated with obesity, including coronary artery disease, type 2 diabetes, certain cancers (7), hypertension, dyslipidemia, stroke, liver and gallbladder disease, sleep apnea and respiratory problems, osteoarthritis, and gynecologic disorders such as abnormal menses and infertility. The underlying causes of obesity represent a complex web of interactions including inherited genetic traits, low physical activity levels, environmental factors such as access to affordable healthy food, cultural identity, socioeconomic status, and others.

At the cellular level, work over the past decade has increasingly linked obesity and inflammation (2). It has been demonstrated that immune cells infiltrate adipose tissue at the onset of weight gain and directly contribute to and perpetuate the inflammatory state of fat, systemic insulin resistance, and the promotion of obesity (8–14). One inflammatory cell, the macrophage, accumulates in obese adipose tissue and quantities directly correlate with adipocyte size, age, female sex, and

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\* To whom correspondence should be addressed. E-mail: liza.makowski@unc.edu.

numerous inflammatory mediators (15). Clear associations exist between obesity, metabolic syndrome, and macrophage-mediated inflammation (16). Classically activated macrophages infiltrate adipose tissue at the onset of weight gain, and contribute to the chronically inflamed state that leads to insulin resistance and diabetes. In contrast, alternatively activated macrophages safeguard insulin sensitivity and tissue homeostasis in metabolically active tissues (2). Many questions remain about how macrophage phenotypes are regulated within the adipose microenvironment along the classical-alternative polarization spectrum. Our group focuses on the relation between the tissue microenvironment and macrophage metabolism in regulating obesity-associated inflammation in diseases such as diabetes, atherosclerosis, and breast and ovarian cancers. This review focuses on the preclinical and clinical considerations of using metabolomics as a tool to aid in understanding how metabolism regulates the inflammatory response.

## How Is Obesity Studied?

Preclinical models are commonly used to mimic human obesity, albeit with the understanding that mice do not always perfectly model human physiology. There are 2 methods for modeling obesity in preclinical rodent-based systems: by using genetic manipulation approaches, such as gene mutation or overexpression, or by using custom diets to induce obesity. The first knockout mouse model was created in 1989 (17). Since then, the use of genetic models in the laboratory where a single gene is deleted or overexpressed is a common method that is widely used for modeling and understanding the etiology and progression of an array of diseases, including obesity. Various model systems have been developed that are aimed at studying the underlying biochemical mechanisms that play a role in the onset and progression of obesity, as well as the metabolic pathologies associated with obesity. Existing mouse models are available through companies such as The Jackson Laboratory or Charles River Laboratories, or models can be made in the laboratory or at many mouse core facilities at most research institutes. Examples of genetic models of obesity include *ob/ob* mice, which lack leptin; *db/db* mice, which lack leptin receptor; Agouti yellow mice; melanocortin 4 receptor-deficient mice; and Zucker *fa/fa* obese rats. Such monogenic approaches have proven to be powerful tools providing abundant information into the contribution of single pathways to obesity. However, they are limited in terms of how this information can be translated to the condition of human obesity because it is exceedingly rare that humans have a single gene mutation that leads to obesity (18). The reality is that obesity is a complex genetic disorder involving multiple single nucleotide polymorphisms, epigenetic patterns, and multiple, cumulative exposures beginning in utero and continuing over an entire life span. The environmental exposures at play include physical activity, environmental chemicals, our microbiome, and importantly, our diet—all of which contribute to obesity onset, progression, and severity.

Although rodent genetic models of obesity have provided valuable insight into the biochemical underpinnings of obesity, for the reasons discussed above diet-induced obesity (DIO<sup>6</sup>) models more accurately represent obesity observed in humans.

Thus, the use of animal models exposed to obesogenic diets best model the human condition (19). There are many diets that cause obesity. Rodents readily consume high-fat diets and can be weaned onto these diets. Obesity onset is usually rapid, with significant weight gain observed within weeks to a few months from the initiation of the diet. DIO experiments can be extremely well controlled due to the ability to use littermates, which ensures that study animals are genetically identical and exposed to similar in utero, maternal, and home cage environments.

Humans consume, on average, 34–45% of energy from fat (20), which must be taken into consideration in the laboratory setting, where scientists aim for the most human-relevant high-fat intake through DIO models. There are several DIO diets that are regularly used, each with its own set of advantages and shortcomings; however, first, it is important to understand unpurified diets, especially when considering the most appropriate “control” for the DIO diet to be used. Rodents in animal facilities are commonly maintained on a feed pellet diet, consisting of ground oats, barley, wheat, and corn (a diet high in fiber and phytoestrogens). Importantly, unpurified diets are undefined, and although the composition of macro- and micro-nutrients in unpurified diets is always constant, the source of those nutrients can vary from lot to lot. Recognizing this fact is important when analyzing and interpreting DIO model data if unpurified diets are chosen as the “control” diet.

Purified or defined diets are diets that are mass produced by companies wherein all diet components are known. Choline-deficient and methyl-donor-deficient diets, which lack choline, folate, and methionine, or high-sucrose diets are 3 obesogenic diets that can be used in DIO studies. However, by far the most commonly used diets are the high-fat diets. The most widely used diet is a lard-based van Heek series, which includes a range of high-fat diets with 45% or 60% of energy derived from fat, typically lard; therefore, these diets have a high saturated fat content. They are commonly paired with a diet with 10% of energy derived from fat (a “low fat” control diet) that does not induce obesity. The pitfall with this 10% control diet is that to be isocaloric to the high-fat diets that contain 45% or 60% of energy from fat, the diet containing 10% of energy from fat is usually high in sucrose, which can induce fatty liver. Another alternative diet is the Western diet, which also derives its fat from lard, as with the van Heek series, and contains added cholesterol to induce atherosclerosis. In addition, specialized diets are available, including the Surwit diet which is high in coconut oil (medium-chain fats), for specific scientific questions. As discussed above, a complicating factor for many of these diets is that to remain isocaloric, sucrose is added to the low-fat diet to match the caloric content of the high-fat diet. Sucrose is a combination of glucose and fructose, which can induce weight gain through multiple pathways (21), although to a lesser extent than high-fat diets. To overcome this limitation, sucrose-free diets are available in which sucrose has been replaced with a more complex carbohydrate such as cornstarch.

Finally, the diet model that may best mimic human obesity is called the Cafeteria (CAF) diet. The CAF diet is a labor-intensive smorgasbord feeding model in which rodents are provided the choice of unpurified diets or highly palatable, calorically dense, nutrient-deficient human “junk” foods purchased from a grocery store. Therefore, the food exposure more closely parallels what humans are eating. Indeed, Piernas et al. (22) reported that in preschool-aged kids, snack foods account for 27% of total caloric intake. Why is this important? Snack foods are energy dense, providing lots of calories with little nutritional value. For

<sup>6</sup> Abbreviations used: CAF, Cafeteria; DIO, diet-induced obesity; FABP4, fatty acid binding protein aP2; GLUT1, glucose transporter 1; IκK, inhibitor of kappa B kinase; RTP, Research Triangle Park, NC.

example, 100 g of fresh corn has 83 kcal, a corn tortilla contains 210 kcal, and fully processed, energy-dense tortilla chips contain 493 kcal per serving.

Our laboratory was interested in comparing the obesogenic and inflammatory effect of various diets including the CAF diet. We provided a choice of 3 human foods that varied from day to day, including cookies, salami, chips, chocolate, muffins, cereals, and cheese, among other options. Detailed information on the diets is available in Sampey et al. (21, 23). Although rats fed this junk-food diet ate ~17% of calories from feed pellets, overall we observed hyperphagia resulting in rapid weight gain in the CAF diet-fed rats above and beyond what we observed in the rats fed diets that contained 45% of energy from fat and 10% of energy from fat and an unpurified diet-only control diet. Rats fed the control diets consumed ~100 kcal/d, whereas rats fed the CAF diet consumed 130–150 kcal/d (21). The CAF diet is a model to broadly mimic human snacking, because a variety of options and the palatability of snack foods lead to overeating (21). CAF diet-fed rats developed prediabetes characterized by elevated insulin, fasting blood glucose, and circulating nonesterified FFAs, with concurrent impaired glucose and insulin tolerance. Both the liver and adipose tissue from CAF diet-fed rats were severely inflamed, and markers of pancreatic islet dysfunction were evident. Of note, when we compared rats fed the low-fat (10% of energy from fat) and high-fat (45% of energy from fat)-defined diets, we did not see significant divergence between the groups at the same time point at which we saw dramatic CAF diet-induced metabolic dysfunction. Given the remarkable effects of the CAF diet, we turned to metabolomics to provide insight into why the CAF diet induced inflammation that was much more severe than the typical 45% high-fat experimental diet. Using metabolomics and bioinformatics analysis, we identified mitochondrial dysfunction and a specific acylcarnitine, lauroylcarnitine, a metabolite of FA oxidation that was elevated in adipose tissue of CAF diet-fed rats compared with high-fat-diet-fed or control rats (21, 23). Lauroylcarnitine significantly correlated with crown-like structures in adipose tissue, markers of inflammation and insulin resistance. We then demonstrated that lauroylcarnitine drove proinflammatory macrophage polarization and cytokine release, therefore providing a potential mechanism linking obesity, lipid metabolism, and inflammation to be tested in future studies (21, 23).

## How Does a Molecular Biologist Incorporate Metabolomics into Nutrition Research?

Substantial progress has been made in understanding the inflammatory phenotype of macrophages in the microenvironment of tissues such as adipose, liver, and the vessel wall in atherosclerosis. However, how metabolism regulates macrophage polarization, thus influencing tissue inflammation and insulin resistance within metabolically active tissue microenvironments, is poorly understood (2). Addressing this question is essential because cellular and molecular pathways regulated by metabolites, such as bioactive lipid mediators, have yet to be explored in great detail and will have tremendous potential as novel therapeutic targets. The ability to harness control of the “immune-metabolic” milieu could break the proinflammatory cycle and would control insulin resistance, compensatory hyperinsulinemia, and hyperglycemia of diabetes. Our previous work on cytosolic FA binding protein aP2 (FABP4) lends support to the concept of fuel metabolism and inflammation being

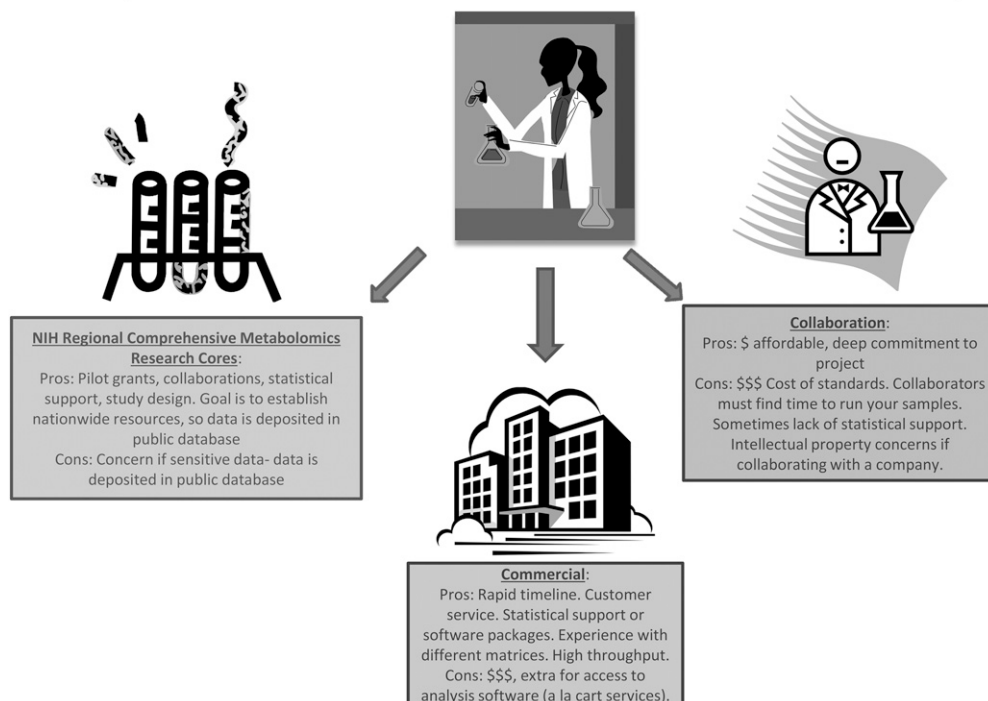
interdependent; we demonstrated that the integration of lipid trafficking and signaling in the macrophage contributes significantly to the pathogenesis of atherosclerosis, insulin resistance, and obesity through multiple pathways such as the nuclear hormone receptor PPAR- $\gamma$ , modulation of reverse cholesterol transport, the inflammatory inhibitor of kappa B kinase (I $\kappa$ B)-NF- $\kappa$ B kinase pathway, and endoplasmic reticulum stress (2, 21, 23–33). Furthermore, Vats et al. (34) established that generation of alternatively activated macrophages is an FA oxidation-dependent process. We recently demonstrated that metabolic reprogramming of macrophages is possible in vitro through overexpressing glucose transporter 1 (GLUT1) (35). Using metabolomics, we showed that forced glucose metabolism and activation of the pentose phosphate pathway was sufficient to polarize macrophages to the classically activated macrophage phenotype (35). Therefore, an important modifier of macrophage plasticity and the inflammatory tone contributing to the onset of diabetes is the availability of fuel substrates to the macrophages in the microenvironment. Biochemists recognize that glucose- and lipid-involving metabolic pathways, in fact all metabolic pathways, work in concert to maintain cellular and tissue homeostasis. Therefore, altering some aspect of glucose metabolism, for example, will undoubtedly have effects on lipid and protein metabolic pathways. Classic radiotracer methods are useful for determining flux through a specific pathway, but to get at the heart of how a cell or tissue is responding to a metabolic change in a more holistic way, metabolomics can be used.

## What Are the Options for Metabolomics for Clinicians or Bench Scientists?

As biochemists who want to understand the metabolic underpinnings of processes but do not have the chemistry, informatics, or programming background to answer such questions alone, we have to turn to others to conduct metabolomics analyses (Figure 1). When designing a metabolomics study, one must carefully consider the hypothesis to be tested or question asked while at the same time designing studies that are sufficiently powered to detect differences considering the expected biological variation and keeping within budgetary constraints. One may conduct targeted investigations to detect a few important metabolites or run samples through multiple platforms in an unbiased discovery-based approach. As with other technologies, cell culture studies are inherently less variable than mouse samples, which are, in turn, less variable than human samples. Controlling for as many variables as possible is imperative. In our experience, 50–200 mg for most tissues, 50–100  $\mu$ L of plasma, or ~1 million cells are sufficient for most metabolomics analyses (23, 24, 35–37). Sample size should be as great as possible because multiple metabolites are measured, which is similar to a microarray study. Clinical considerations include sample size and proper controls as in any study; however, one must consider the metabolic state of patients (fasted, taking medications, etc.) and the time to capture the sample from the operating room or clinic to the freezer. Some studies use a “stop watch” approach and place a stop watch with the sample when the clinician isolates it. The stop watch only gets turned off when the sample is frozen in liquid nitrogen. Depending on the isolation of the sample, availability of the research associate, and transport to the laboratory, sample freezing times can vary from minutes to many hours. This is highly relevant when analyzing metabolites. Furthermore, because clinical samples are precious, it is best to

# METABOLOMICS OPTIONS

**FIGURE 1** The pros and cons of metabolomics.



optimize on practice samples. Finally, a benefit of using metabolomics rather than other biomarkers (e.g., serum or urine proteins such as a cytokine) is that metabolites do not vary by species. There can be extensive cross-species validation.

There are several approaches for initiating metabolomics research—with pros and cons for each—and every laboratory must decide what fits best. First, for those “do it yourself-ers” with the proper machinery and training, metabolomics is a matter of having the right authentic standards to quantitate specific metabolites of interest and the right people to prepare samples and operate your machines. One caveat to conducting

your own metabolomics is that it is often cost-prohibitive to purchase the hundreds of authentic standards necessary to conduct comprehensive discovery work. Furthermore, without the proper training in programming and statistics, one is often left “eye-balling” the data for significant findings. Of course, there are plenty of outstanding researchers who have excellent metabolomics facilities. Collaborating with such experts is the second route to pursue metabolomics research as a novice.

As with any collaboration, there are pluses and minuses: one must discuss costs, personnel, and authorship upfront. A typical hurdle with collaborations is the timeline for study completion

**TABLE 1** Location and representative publications for sites conducting metabolomic analysis<sup>1</sup>

Commercial sites for metabolomic analysis			
Name	Location	URL	Representative publications
Metabolon, Inc.	Durham, NC (RTP)	www.metabolon.com	(23, 36, 38)
Lipomics, Inc. (owned by Metabolon, Inc.)	Sacramento, CA	www.lipomics.com	(24)
Metabolomic Discoveries	Potsdam-Golm, Germany	www.metabolomicdiscoveries.com	(39, 40)
Chenomx, Inc.	Edmonton, Canada	www.chenomx.com	(41, 42)
RCMRCs, NIH-funded and Common Fund’s Metabolomics program			
NIH West Coast Metabolomics Center at UC Davis (WC3MRC)	Davis, CA	www.metabolomics.ucdavis.edu	(43, 44)
Michigan Regional Comprehensive Metabolomics Research Core (MRC)	Ann Arbor, MI	www.mrc2.umich.edu	(45, 46)
NIH Eastern Regional Comprehensive Metabolomics Resource Core at RTI International (RTI RCMRC)	Durham (RTP), NC	www.rti.org	(47, 48)
Southeast Center for Integrated Metabolomics at University of Florida (SECIM)	Gainesville, FL	www.secim.ufl.edu	(49, 50)
Resource Center for Stable Isotope-Resolved Metabolomics at University of Kentucky (RC-SIRM)	Lexington, KY	www.bioinformatics.cesb.uky.edu	(49, 50)
Metabolomics Resource Core at the Mayo Clinic	Rochester, MN	www.mayo.edu	(51, 52)

<sup>1</sup> RCMRC, Regional Comprehensive Metabolomics Research Core; RTP, Research Triangle Park, NC.

and the possible extended wait time for your samples to be analyzed. However, working closely with experts will help with experimental design, data interpretation, and planning of further experiments. Collaborating with a company that conducts metabolomics is also an option, but that has drawbacks as well, such as access (because not every company will want to collaborate with you) and intellectual property concerns. For example, the method of processing samples used may be proprietary and there may be substantial hurdles to clear when it is time to publish.

The third option is a fee-for-service through companies that specialize in metabolomics such as Metabolon, Lipomics (recently acquired by Metabolon), Metabolomic Discoveries, and Chenomx, among others (Table 1). There can be substantial costs associated with this approach depending on the service package purchased; services offered can range from study design and sample prep to statistical analysis, data interpretation, and figure preparation. Through their high-throughput capacity and standard operating procedures, companies often will have established protocols for your samples, including quantities necessary, tricks for preparing samples, etc., that save time and money because assay optimizations are not necessary. Because it is a fee-for-service rather than a collaboration, data are usually generated within an established time as agreed upon when a contract is signed. Companies must consider customer satisfaction and repeat business, but this is a relationship that you pay for. Although you are guaranteed a final product, the option to go back and conduct follow-up experiments is not possible without acquiring additional costs.

A fourth and final metabolomics option is a new initiative supported by the NIH funded through the Common Fund's Metabolomics program (Table 1). This is a national program aimed at increasing metabolomics capacity by developing centers with high-throughput technologies, training, and mentoring for metabolomics researchers. The goal is to standardize protocols in an effort to increase sensitivity and speed to identify metabolites. In addition, by providing reference standards and data sharing, the NIH hopes to build a publicly accessible national database that will propel metabolomics research forward. There are currently 6 Regional Comprehensive Metabolomics Research Cores (RCMRCs) funded by the Metabolomics program. The cores have expertise, instruments, and training programs to increase metabolomics capacity. Existing sites are as follows: 1) NIH West Coast Metabolomics Center at UC Davis (WC3MRC), 2) Michigan Regional Comprehensive Metabolomics Research Core (MRC), 3) NIH Eastern Regional Comprehensive Metabolomics Resource Core at RTI International (RTI RCMRC), 4) Southeast Center for Integrated Metabolomics (SECIM), 5) Resource Center for Stable Isotope-Resolved Metabolomics (RC-SIRM), and 6) the Metabolomics Core at the Mayo Clinic.

Importantly, with strong bioinformatics support, "omics"-based approaches can be used for hypothesis generating, biomarker identification, and, ideally, lead to biomarkers in vivo or in vitro to be tested as bona fide biomediators of observed effects. In summary, the link between metabolism and inflammation is an understudied phenomenon that has great potential to transform our understanding of immunometabolism in obesity, diabetes, cancer, and other diseases. One important benefit of metabolomics is that metabolites are conserved across species, as mentioned above, which makes it easy to translate preclinical findings to humans. Bioinformatics approaches provide a deeper understanding of the complex relations between these pathways that would otherwise be impossible

with traditional biochemical assays. Current and future students and postdoctoral fellows should be encouraged to incorporate statistical training, computer programming, and informatics into their education in conjunction with basic molecular biology techniques and a strong understanding of metabolism to best be prepared for the next era of high-density, high-throughput data generation using metabolomics.

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