Obesity

Low Macrophage Content in Diabetic and Aging Human Skeletal Muscle

Dongmei Liu and Paul M. Gordon

TO THE EDITOR: The prevailing viewpoint is that the accumulation of macrophages in adipose tissue plays a major role in obesity-associated inflammation. However, an increase in skeletal muscle macrophage content and subsequent paracrine effect has also been implicated in the pathogenesis of muscle insulin resistance (1,2). Yet, other studies have found minimal amounts of macrophages in skeletal muscle and no relation to adiposity levels (3). The discrepancy in these findings may be due, in part, to different methods used for determining macrophage contents. For example, in obese human skeletal muscle, Varma et al. used immunohistochemistry staining for CD68 and found an increase in macrophage count (1); whereas, Bruun et al. used real-time polymerase chain reaction (RT-PCR) to measure CD68 mRNA levels and reported minimal macrophages that were unchanged following weight loss (3). To clarify the role of local macrophage in muscle insulin resistance in obesity and diabetes, studies using multiple methods to ascertain the quantity of macrophages are needed. A recent short communication by Tam et al. (4) represents such a study, in which both immunohistochemistry and gene expression were used to assess muscle macrophage content in obese diabetic and elderly individuals. Tam et al. reported little evidence of macrophage accumulation in muscle of obese diabetic (2–3%) or elderly (~4%) individuals, based on immunohistochemistry staining for CD68. This was much lower than that observed by others who used similar methods and reported approximately 25% macrophages in the muscle of obese (1) and elderly (5) individuals. Tam et al. suggested that the reason for higher macrophage levels found by others might be due to contamination by adipose tissue, given that they observed greater CD68 staining in the intermuscular adipose tissue (IMAT) region of obese diabetic muscle. Tam et al. chose to exclude connective tissue and IMAT regions in the muscle sections used for macrophage counting, which we find concerning. Connective tissue and IMAT surrounding myofibers likely influence myofiber function. As suggested by Kewalramani et al., macrophages infiltrating muscle tissue, whether bordering the myofibers directly or surrounding muscle-infiltrating adipocytes, might have a large impact on muscle insulin activity (6). This notion is supported by *in vitro* studies, where conditioned medium collected from palmitic acid-treated macrophages resulted in a significant decrease in insulin-mediated glucose uptake in both cultured human muscle myotubes (1) and L6 myotubes (7).

Animal studies indicate that resident macrophages are extensively present in the connective tissue surrounding myofibers (8). Moreover, enlarged IMAT likely attracts monocyte-derived macrophages (9). Therefore, we feel that connective tissue and IMAT are important regions to examine for abnormalities in muscle macrophages. Excluding these compartments of muscle tissue could lead one to overlook the potential contribution of local macrophages to muscle metabolic dysfunction in obesity and aging.

Additionally, we are curious about the changes in macrophages following the exercise intervention in the diabetic patients. Although no significant change was reported, there appears to be an increase in macrophage content in at least half of the subjects based on the median and interquartile ranges at baseline 2.7 (2.0–3.2%) and postintervention 4.0 (3.2–6.8%).O

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Better Measurement Needed to Move Food-Environment Research Forward

Sean C. Lucan and Earle C. Chambers

TO THE EDITOR: Dubowitz et al. provide the latest data linking select aspects of local food environments to select obesity-related health measures (1). The authors' lengthy limitations section highlights some of the measurement challenges to conducting this type of research. If future research is to identify the most promising strategies to improve food environments, support healthy eating, and prevent obesity, then the field needs to move beyond some common measurement limitations of early studies.

First, the use of unvalidated business-list data is precarious. Although such data were

convenient and efficient for establishing foundational associations in early studies, they are less than ideal for the field moving forward. For example, based on detailed ground observations of all food sources from >150 Bronx streets, research in our group has shown the business list used by Dubowitz et al to have a positive predictive value below 50% overall, and a sensitivity for grocery stores of only about 25% (manuscript under preparation). Other researchers have reported similar findings (and the business list having a sensitivity of <10% for fast food) (2). More-accurate food-environment data, verified on-the-ground, are needed.

Beyond issues of accuracy, the concern with reliance on business lists is two-fold: [1] it inappropriately groups all food sources of a certain type (e.g., as if every supermarket were the same as every other supermarket in terms of selection and quality of products, when empirically this is not the case (3)), [2] it generally restricts focus to a limited range of food outlets (e.g., ''fast-food restaurants,'' ''grocery stores and supermarkets'' (1)). Studies using business-lists necessarily neglect impermanent sources of food that may be relevant, like street vendors (4,5) and farmers' markets (6,7). They also routinely neglect–more due to investigator decision as opposed to data deficiency a range of other potentially relevant food sources like pharmacies, gas stations, hotels, salons, general merchandisers, clothing outlets, book stores, and other retail selling food and drink (8). More-complete availability measures, that include generally neglected food sources, are needed.

A separate issue concerns definitions of food-source proximity. While studies like that of Dubowtiz et al commonly use radial ''buffers'' (1), such buffers ignore how people travel in their environments and their barriers to transit. For example, one-way streets and divided highways have different implications for motorists than for pedestrians, and research that fails to consider how people actually access food may miss relevant associations (9). An alternative approach to radial buffers, favored by geographers, is to measure accessibility along existing street networks for walking or driving (10). More-precise accessibility measures, that consider how people travel to get food, are needed.

Dubowitz et al. conclude that their findings support restricting the development of fast-food outlets and attracting grocery stores (1). Given measurement limitations, including those above, we do not find strong support for this conclusion. Perhaps greater benefit would come from promoting famers' markets and produce carts … or from restricting the sale of unhealthy foods at salons and pharmacies (but maybe only for those along walking routes and not for those accessed by motor vehicles). To investigate such possibilities and understand different options for intervention, it is time for the field to move beyond the limitations of early research, making use of more accurate, complete, and precise measures.

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Re[illegible] Evidence to Support a Beneficial Role for Glutathione Depletion on Body Weight or Glucose Intolerance''

Hannes M. Findeisen and Dennis Bruemmer

TO THE EDITOR: We appreciate the concerns shared by Dr. Sekhar regarding our recently published manuscript. With respect to the title of the letter to the Editor by Dr. Sekhar ''Lack of evidence to support a beneficial role for glutathione depletion on body weight or glucose intolerance'' it appears pertinent to first summarize the available literature that has addressed the role of glutathione depletion in obesity and metabolism. Abundant evidence has now revealed that mice genetically deficient for glutathione or glutathione peroxidase are resistant to weight gain and diet-induced obesity, are protected from glucose intolerance and insulin resistance, do not develop hepatic steatosis, and exhibit a higher metabolic rate (1–4). Therefore, there is indeed unequivocal evidence that glutathione depletion elicits a phenotype in mice that is beneficial for body weight regulation and glucose homeostasis. Moreover, our data using a well-established pharmacological model of glutathione depletion are entirely consistent with this evidence obtained from genetic models. Due to the nature of our article being a short communication and considering the extensive literature describing buthionine sulfoximine (BSO)-induced glutathione depletion in rodents (5,6), we did not show glutathione reduction in our initial manuscript. However, we found a reduction of almost 70% in total glutathione levels in epididymal adipose tissue of BSO treated mice ($P < 0.05$). The extent of glutathione reduction in response to BSO in our studies is similar to that previously seen in pharmacologic or genetic models of glutathione depletion (2,5,7).

With respect to the second comment on the measurements of food intake, these experiments were performed following the guidelines set forth by the National Institutes of Health (NIH)-funded Mouse Metabolic Phenotyping Centers (8). Specifically, we employed a calorimetry system that provided investigator-independent automated feeding systems. Food intake was measured

cumulatively during a 72 h time period and expressed as gram food consumption per day. Our observation that BSO-treatment did not affect food consumption is, again, in line with various previous publications demonstrating normal food intake in BSO-treated mice (5,9) as well as with the above-described genetic models of glutathione depletion (2). Nonetheless, we agree with Dr. Sekhar that further comprehensive studies to define this intriguing phenotype are warranted, including particularly pair-feeding experiments.

Finally, Dr. Sekhar expresses concerns about possible toxic effects of BSO. We have elected to treat mice with a dose of 30 mM BSO because this dose has been previously demonstrated to effectively decrease glutathione in mice (7). Moreover, extensive characterization of various organ functions in mice treated with this dose, including liver function tests and cytochrome P-450 concentrations, revealed no toxic effects after prolonged treatment (7). In our study, high fat diet fed mice exhibited a significant increase in liver weight during the course of the study, an effect likely secondary to steatosis. In contrast, the development of this phenotype was prevented in BSO-treated mice (data not shown). This observation is consistent with data obtained in mice that are genetically deficient for glutathione and has been attributed to a specific beneficial effect of glutathione depletion on the expression of lipogenic genes (2,3,6). Consequently, it is not surprising that the authors of the study referred to by Dr. Sekhar (9) also noted an effect of BSO on liver weight. However, in this study Watanabe et al. (9) did not observe any avoidance of food/water intake, and the authors did not interpret 30 mM of BSO as hepatotoxic, considering that BSO did not increase alanine transaminase (ALT), alkaline phosphatase (ALP), or the expression levels of drug-metabolizing pathways (albeit a modest isolated increase in aspartate transaminase (AST) levels). Collectively, these studies do not support the assumption that the dose of 30 mM BSO used in our studies elicited toxic activities that contribute to the beneficial obesity and metabolism phenotype.

In summary, our manuscript supports a more complex role of glutathione in the regulation of energy balance and metabolism than previously anticipated. We acknowledge that our manuscript, collectively with the discussed literature, challenges the conventional hypothesis that depletion of glutathione may be detrimental for obesity and metabolism. Determining the molecular mechanisms underlying these phenotypes and potential pathological consequences constitute important avenues for future research. O

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Response to Low Macrophage Content in Diabetic and Aging Human Skeletal Muscle

Charmaine S. Tam[1,2], *Jeffrey D. Covington*[1] *and Eric Ravussin*[1]

TO THE EDITOR: The pro-inflammatory role of macrophage accumulation in white adipose tissue in obesity is well established and may be one of the causes of insulin resistance. While [illegible] in skeletal muscle [illegible] inflammation state and insulin resistance is less clear. We therefore measured inflammation gene expression and CD68+ macrophages in skeletal muscle from obese subjects with type 2 diabetes mellitus before and after a 9-month exercise intervention (1). In contrast to previous studies in similarly obese subjects (2,3), we found relatively few macrophages (2-3%) and low inflammation gene expression (CD68, CCL2, CD40, CD206, CD11c, Arginase 1) in obese skeletal muscle, which was unchanged after exercise training (1).

We examined skeletal muscle sections, deliberately excluding areas of connective tissue and adjacent adipose tissue, and concluded that greater macrophage accumulation in other studies may potentially be due to contamination with adipose tissue. In response to our report (1), Liu and Gordon pointed out that connective tissue and inter-muscular adipose tissue (IMAT) may be important regions to examine macrophages in skeletal muscle (4). Indeed, recent studies in subcutaneous and omental adipose tissue in obese humans have demonstrated that connective tissue regions (fibrotic areas within adipose tissue) contain macrophages (M1 and M2 phenotype) and mast cells, with few T lymphocytes (5). Spencer et al. found higher macrophage accumulation in fibrotic areas in obese compared to lean subjects (6).

Together, these studies indicate potential cross-talks between inflammatory cells in fibrotic areas and the adipocyte, which may further exacerbate obesity-associated low grade inflammation and potential insulin resistance (5,6). As pointed out (4), it is possible that immune cells may also be present in connective tissue areas in skeletal muscle, as shown in rodents (7) but yet not in humans. Interestingly, when we re-examined our skeletal muscle sections, we saw few to no areas of connective tissue (data not shown).

Liu and Gordon also suggest that macrophages may be present in IMAT which is located between muscle bundles and clearly separated from subcutaneous adipose tissue (8). Magnetic resonance imaging studies demonstrate that such fat depots are approximately twofold higher in subjects with obesity and type 2 diabetes (8). Furthermore, *in vitro* co-culturing of human myotubes and adipocytes results in impaired insulin action depending on the metabolic state of the system (9). Unfortunately, IMAT depots are

difficult if not impossible to obtain from muscle biopsies. In addition, in response to the authors' comments, we re-examined the raw data and noted that 5 out of the 7 subjects had relatively small increases in macrophage accumulation after exercise training.

In summary, there is a need for further investigations examining inflammation in skeletal muscle in the context of obesity and type 2 diabetes and its potential role in mediating insulin resistance. *In vitro* studies using palmitate treated macrophages have demonstrated impaired insulin-mediated glucose uptake in cultured human (2) and L6 myotubes (10). Exploring immune cells in areas of connective tissue and adjacent adipose tissue clusters in skeletal muscle from obese subjects may provide further insights into muscle inflammation.

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Food Policy Research: We Need Better Measurement, Better Study Designs, and Reasonable and Measured Actions Based on the Available Evidence

Tamara Dubowitz[1], Madhumita (Bonnie) Ghosh-Dastidar[2], Rebecca Collins[3] and José Escarce[4]

TO THE EDITOR: Lucan and Chambers, in their Letter to the Editor, call for better measurement in order to move food-environment research forward. We wholeheartedly agree. In fact, this issue is not new to the obesity policy agenda (1). However, attaining validated and detailed food environment data in a large-scale (e.g., national) setting is prohibitively costly. Our study included 68,132 women living in 18,186 census tracts across the United States (2). We excluded women who lived in census tracts with a population count of less than 500 and women living outside metropolitan statistical areas because we believed measures of the food environment would not be comparable in urban and rural areas. As we pointed out in our article, most studies to date have analyzed a single type of food outlet (e.g., grocery stores or fast-food outlets) at one time. We examined multiple dimensions of the food environment in a national dataset—and believe that these data and analyses bring the state of the literature forward. We concur that detailed ground observations, such as the ones Lucan and Chambers reference, are ideal. However, these can only be executed in confined geographic settings. Such data would be extremely difficult, if not impossible, to attain on a national scale.

We also agree with Lucan and Chambers' concerns with (1) assumptions that establishments categorized as full-service supermarkets are all comparable; (2) potentially relevant food sources such as farmers' markets and mobile produce stands may not be captured through commercial database listings; and (3) [illegible] a store [illegible] capture [illegible] factors such as transportation mode, travel time, and social [illegible] food purchasing. Such detailed evidence can complement and validate studies based on large national data to ensure that policies are based on a solid scientific foundation.

Importantly, we are working on that. Members of our team are involved with the largest study to date in the United States that is capitalizing on a natural experiment of the elimination of a food desert (1R01CA149105, *Does a New Supermarket Improve Dietary Behaviors of Low-income African Americans?*). Examination of a natural experiment of this type (i.e., elimination of a food desert) is allowing our team to overcome many of these limitations, from reliance on unvalidated commercial databases (we are conducting food audits to collect price, quality, and availability of food data from *all* food purchasing venues in residential neighborhoods included in our study as well as the most frequently report venues our enrolled population reports shopping), to a longitudinal quasi-experimental study design with a control or comparison neighborhood, and extensive data on residents' dietary intake, travel mode, time spent in shopping, and experience of food purchasing. We have just completed our baseline data collection and hope that findings from this study when completed will be replicable to other low-income African American neighborhoods across the United States. However, we are indeed confined to one large natural experiment and unlike our published study, will not be analyzing data based on tens of thousands of individuals and census tracts.

We agree that the field faces measurement limitations. Large observational studies, such as the one our paper reported, have imperfect measures. However, given the considerable impact of nutrition on obesity and other health problems, we believe that reasonable and measured actions based on the available evidence need to be considered. Policy makers cannot afford to rely solely on data from detailed studies of a few neighborhoods (one could argue a requirement for grounding approaches) and need to know whether results hold at a national level. Our study does that, and the methods we used are necessary to such a study. Although we agree that the field also needs studies of small areas with rich and detailed measures to complement the national data

and help us determine their validity, we still conclude that our findings support restricting the development of fast-food outlets and attracting grocery stores, and are committed to additional research that overcomes the limitations of large studies such as the one we published.

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Adolphe Quetelet and the BMI: Fact, Fiction, and Childhood Growth

Richard F. Burton

TO THE EDITOR: It is often stated, with or without supporting references, that the BMI was proposed by Adolphe Quetelet (e.g., see Refs. 1,2). However, I find no evidence for this in his own writings, of which I cite only three here (3–5). What he did assert was that body mass in adults tends to vary with the square of height (3–5).

Less often noted is that he also wrote that mass^2 increases with height^5 in adolescents (3–5), implying that a more appropriate version of the BMI for them is not mass/height^2, but mass/height$^{2.5}$. This accords approximately with values of p in the Benn Index (i.e., mass/height^p) tabulated in a notable paper by Cole for successive one-year age groupings (6). (That height and mass were standardized for age is unimportant here.) Estimates of p were generally 2.2-3.3 in children between four and about 15 years of age. The highest values tended to occur around the time of puberty. None significantly exceeded 3.0, which is the value Quetelet recognized as appropriate to isometric growth (3–5).

The variation in p largely relates to variation in the correlation coefficient, r_{MH}, for the logarithms of mass-for-age and of height-for-age. Cole's Table 2 reveals a marked correlation between the values of p and r_{MH} in both boys and girls from the age of six ($n = 40$; $r = 0.89$; $P < 10^{-10}$). This recalls a similar correlation between estimates of p and the respective correlation coefficients for mass and height in adults (7). In the children, again from the age of six, the 40 values of r_{MH} correlated with the standard deviations of log(height-for-age) ($r = 0.55$; $P < 0.0002$). Thus, in those age groups in which the adolescent growth spurt makes height most variable, r_{MH} tends to be highest and p tends to be closest to 3.

The correlations between p and r_{MH} in adults and children are inherent in the statistical techniques used in estimating p (7). With adequate samples, the estimates that they produce will always be less than the true (functional) values. It means that Quetelet's assertion and continuing common belief, that adult body mass tends to vary with height^2, is actually wrong (though this does not invalidate the BMI as a predictor of %adiposity in adults). The apparent allometry in adults is largely or entirely a statistical artifact. The childhood peak in p is also largely or entirely a statistical phenomenon, explainable in terms of growth, but not of changing bodily proportions such as might relate to adiposity. The statistical reasoning is uncommon in anthropometry and can seem counterintuitive, but it is readily tested by Monte Carlo modeling (7).

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Single Slice vs. Volumetric MR Assessment of Visceral Adipose Tissue: Reliability and Validity Among the Overweight and Obese

Siamak Sabour, MD, PhD

TO THE EDITOR: I was interested to read the paper by Maislin et al. published in the May 2012 issue of *Obesity*. The authors assessed reliability and validity of single slice vs. volumetric MR of visceral adipose tissue (VAT) among the overweight and obese and reported that the correlation with VAT volume was significantly larger for L2-L3 VAT area ($r = 0.96$) compared to L4-L5 VAT area ($r = 0.83$) (1). These correlation computations do not reflect reliability and validity analysis and is a common mistake in reliability analysis (2-4). I found the manuscript title of Maislin et al. incorrect and misleading. Moreover, they reported a strong positive correlation between variables in both areas; clinically 0.13 differences in r means nothing, although it was statistically significant. As a rule of thumb in clinical epidemiology, clinical importance should be considered a priority instead of statistically significant. The P value can easily be changed from significant to non-significant due to small sample size, the amount of mean difference, and more important factor which is standard deviation of the variable in the study population (2-4). As the authors point out in their conclusion, linear regression analyses demonstrated that L2-L3 area alone was sufficient for predicting total VAT volume. The common practice is to employ two different sets of cohort data for

developing and validation of a prediction model, and it is unclear why the authors did not consider employing such practice. The authors also did not utilize Area Under the Curve (AUC) analysis, which would have added diagnostic value to the study (2-4).

Reliability and validity are two completely different methodological issues in researches. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), likelihood ratio positive (LR+: true positive/false positive) and likelihood ratio negative (LR−: false negative/true negative) as well as odds ratio (true results/false results—preferably more than 50) are among the tests to evaluate the validity (accuracy) of a single test compared to a gold standard (2-4). Reliability (repeatability or reproducibility) is being assessed by different statistical tests such as Pearson *r*, least square, and paired *t* test which all of them are among common mistakes in reliability analysis (5). Briefly, for quantitative variable intra class correlation (ICC) coefficient and for qualitative variables weighted kappa should be used with caution because kappa has its own limitation too. Regarding reliability or agreement, it is good to know that for computing kappa value, just concordant cells are being considered, whereas discordant cells should also be taking into account in order to reach a correct estimation of agreement (weighted kappa) (2-4). It is crucial to know that there is no value of kappa that can be regarded universally as indication of good agreement. Statistics cannot provide a simple substitute for clinical judgment. Two important weaknesses of *k* value to assess agreement of a qualitative variable are as follow: It depends on the prevalence in each category and also depends on the number of categories. So it is obvious that the less our categories, the higher will be our kappa value which can easily lead to misinterpretation (2-4).

Area under the curve (AUC) is usually reported for diagnostic rather prognostic values of a model. The Receiver Operative Curve (ROC) for models may be comparable with LR^+ for a test because both of them actually use sensitivity and 1-specificity; however, in LR^+ they are divided and in the ROC we should plot sensitivity to 1-specificity. As a take home message, for reliability and validity analysis, appropriate tests should be applied. O

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