

Using salivary biomarkers to identify children at risk of Type 2 diabetes



“The hypothesis is simple. If obesity in children could be prevented, the incidence of Type 2 diabetes in adults would likely be significantly reduced.”

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As the world struggles to control the epidemic rise of Type 2 diabetes (T2D), the principal focus has been on the catastrophic effects of the disease in adult populations [1]. However, the solution can clearly only be realized with effective prevention, which means understanding and controlling the events that lead to T2D in children. Of all of the causative metabolic events requiring control in children, obesity ranks highly [2]. The hypothesis is simple. If obesity in children could be prevented, the incidence of T2D in adults would likely be significantly reduced. This will require identifying children who are at risk of becoming obese well before their BMI for age rises above age norms. Biomarkers of metabolic health and immunometabolism, such as adiponectin, leptin and resistin, may play a key role in this identification process. For example, a recent proteomic analysis of fasting serum samples from prepubertal children who were either obese (before and after weight reduction) or lean control children found that insulin resistance was associated with changes in the degree of expression of specific isoforms

of proteins related to inflammation and metabolism [3].

One inherent limitation to these types of biomarker studies is the difficulty in obtaining blood specimens from a large, representative sample of children. Indeed, the incidence and severity of adverse reactions associated with drawing blood samples in children is not commonly appreciated. In a study of 12,761 screenings of 11–13-year-old children involving intravenous blood samples [4], a total of 256 adverse events (2%) were reported. Most of these adverse events were mild (swelling, hematoma, dizziness, upset stomach, excessive crying, headache, pain, feeling of hotness or coldness, clammy feeling, difficulty breathing, shaking, weakness, dry throat, twisted ankle, numbness, color change in lips, urination). However, 35 instances of loss of consciousness were reported (0.3%) and one suicide occurred, although the Data and Safety Monitoring Board chairman determined that it was not related to the study. As a precaution, on-site emergency medical personnel were maintained during blood draws.

KEYWORDS

- adiponectin • adverse reactions
- C-reactive protein • insulin • saliva

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Although some may consider nonfatal adverse events occurring at a frequency of 0.3–2% as reasonable and acceptable, we must consider parental consent as a major factor in enrolling for such studies. Parents may well ask themselves if it is fair and reasonable to submit their child to this trauma for the sake of a study that offers no immediate benefit to the child. Furthermore, the investigator must ask themselves if the added expense of emergency monitoring can be addressed. As a result, it becomes difficult to conduct large-scale studies in children that require blood sampling. Therefore, if researchers in the field rely solely on blood sampling studies, many unanswered questions will likely remain with respect to the etiology and control of metabolic disease in children.

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We recently developed a noninvasive approach to study potential biomarkers of obesity and T2D in a population of 10–12-year-old Kuwaiti children [5,6]. All of the patients attended public schools, which enroll only native Kuwaiti children. Because per-capita income in Kuwait is high, the socio-economic level of these children is close to the highest in the world. The diet of Kuwaiti children is in many ways similar to that of the USA due to Westernization and access to fast-food establishments. In our study, the concentrations of a panel of 20 hormones and cytokines were assayed in saliva samples. We also collected data on height, weight, blood pressure, heart rate, waist circumference, fitness (step test) and oral health by examination. Family medical history, sleep and nutrition data were collected via patient self-report through a dedicated electronic tablet application [6].

Of the 8319 children enrolled, 26.5% were obese [5]. In this population, none were known to have T2D. We found three salivary biomarkers that provided strong associations with the development of obesity: insulin, C-reactive protein (CRP) and adiponectin. Leptin was also significantly elevated, but salivary concentrations were so low as to constitute an analytic difficulty. Among the obese children in the study, 50% had salivary insulin concentrations >128 pg/ml (~67 pmoles/l or 11 mU/ml in plasma). Whatever the cause of presumptive hyperinsulinemia in these children, their elevated insulin levels may have caused reduced glucose levels, leading to increased appetite. This, in turn, could contribute to their obesity. We also found that 76% of the obese children had salivary CRP values >219 pg/ml. In view of the cardiovascular

consequences of elevated CRP, these children would seem to have an elevated cardiac risk status in addition to being obese. Adiponectin was significantly decreased in a small proportion (10.8%) of obese children with low cardiovascular fitness who did not have elevated CRP or insulin. It has been well recognized that reduced adiponectin correlates with obesity [7] and cardiovascular disease [8] in adults. The divergence of biomarker characteristics between different groups suggests that obesity may have different causation in different groups of different children and may possibly require different treatments.

As a result of the noninvasive nature of this study, we were able to create a ‘fun’ atmosphere. By self-report, 65% of the children found it fun, while 1.6% found it difficult [UNPUBLISHED DATA]. At the same time, we were able to retrieve meaningful data. The noninvasive methodology was quite safe and easy to use. Saliva samples were collected at a rate of approximately 100 samples/h, without one single adverse reaction [UNPUBLISHED DATA].

Although promising, many caveats currently exist to the measurement of biomarkers in saliva. For medical screening purposes, whole saliva is the only reasonable form to be used. Yet, whole saliva is a mixture of many substances: excreted fluid from all salivary glands, food that is eaten, liquids that are drunk and any oral disease products released into the oral cavity. It is possible to collect glandular saliva from the parotid or sublingual salivary glands, but these methods require considerable experience and technical finesse, such that their collection defeats the fundamental utility of saliva as a simple diagnostic sample. Whole saliva samples present many technical problems for analytical measurements. Most, but not all, molecules found in plasma find their way into saliva. If they do, they usually suffer considerable dilution so that the saliva analyst must use the most sensitive methods available. In addition, saliva has variable viscosity, which can make accurate pipetting difficult, often requiring dilution with water and further loss of sensitivity. Then there is the matter of particulate matter, which is always present in saliva. The oral epithelium continuously desquamates into the saliva and often represents about 20% of the whole saliva volume. Coping with the particulate matter usually requires that a low-speed centrifugation step be included in sample preparation before assay. Last, saliva is not sterile; it has more than 100 million bacteria per milliliter, and these can metabolize molecules of interest

or can produce substances that interfere with analytical assays. As a result, plasma or serum values and saliva levels often carry a low correlation coefficient simply because there are many variables that cannot be completely controlled.

What, then, is the promise of salivary diagnostics for obesity and T2D research? There are three domains for potential use of salivary diagnostics: research, clinical and home. The research use is most straightforward. For example, one may wish to determine if alternate diets, certain exercise programs, drugs or nutraceutical therapies are effective for reducing CRP and insulin or for increasing adiponectin in order to reduce the untoward effects modulated by changes in these biomarkers. A saliva biomarker experiment would provide that information. The scientist would simply power the study to take into account the expected variability. Clinical use is a bit more difficult, since higher variability would result in more false-positive and false-negative responses. Our experience is that with well-selected salivary diagnostics, sensitivities of 75–85% are common. For this reason, the best clinical use would likely be for screening or with patients who will not, or cannot, provide blood samples. Use in the home would require a simplified form such as an insulin ‘litmus paper’ test. This is currently within existing technology [9]. Development of this approach would provide capability for early warning and motivation for monitored lifestyle changes. One example of a lifestyle change could be biofeedback therapy, which appears to have some success [10,11].

In the final analysis, saliva provides a sample obtained without trauma, but is admittedly

more difficult to measure and somewhat restricted in the number of substances that can be measured. In research involving children, this may be preferred. In contrast, blood provides a sample that is obtained with some anxiety but is unrestricted and relatively easy to measure. Both have their place. If we want to precisely determine whether or not a myocardial infarction has occurred, a blood diagnostic is clearly preferred. If we want to know whether our CRP or insulin level is reduced by going regularly to the gym, or if we want to know these values in children who are uncomfortable providing blood samples, a salivary diagnostic is the better match.

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Financial & competing interests disclosure

J Max Goodson has filed a provisional patent application on the diagnostic potential of these salivary biomarkers which has been assigned to the Forsyth Institute. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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