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REVIEW

SALIVARY GLANDS AND ADIPOBIOLOGY

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Abstract

This review explores the functional relationships between salivary glands and adipose tissue. Since salivary glands, and in particular the submandibular glands, exert profound systemic effects on organs and inflammatory responses outside the gastrointestinal tract, the question arises if these glands also impact the body's physiological response to increases in adipose tissue deposition and secretion. And, if the adipose tissue deposition and secretion impact the salivary gland's physiological response. To date the evidence is relatively weak that salivary glands significantly impact obesity, or that their function is dramatically altered by obesity, and that the measurement of metabolic peptides in saliva will lead to diagnostic and treatment strategies for obesity and related cardiometabolic diseases. Although obesity detrimentally impacts oral health causative linkages and associations have not been conclusively made between periodontitis and obesity. The most intriguing connections between adipobiology and saliva (or salivary glands) have emerged from unexpected quarters. It was recently reported that adiponectin, resistin and visfatin (adipose tissue-derived signalling proteins collectively termed adipokines) are found in saliva and that their amount correlates with that of circulating level of these adipokines. These observations suggest that the introduction of salivary determinations of adipokines may contribute to the study of pathogenesis of various obesity-related diseases. Receptors for adipokines and obesity-related hormones, especially for polypeptide Y (PYY(3-36), in the mouth and in particular the taste buds, may be a primary signal for satiety. This observation offers new avenues for investigating the physiology of satiety along with potential treatment strategies for obesity. Another unexpected finding, and to date unrelated to obesity – the transplantation of adipose-derived stromal cells has the potential to restore salivary gland function after their destruction by radiation therapy.

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Background

The major salivary glands are the parotid, submandibular, and sublingual glands, although numerous (>600), minor salivary glands are found within the submucosa and mucosa of the lips, inner cheek area, and other parts of the oral cavity. Von Ebner's glands, another salivary gland also called gustatory glands, are found in circumvallate papillae of the tongue and facilitate lipid hydrolysis and taste. The salivary glands make saliva a complex mixture of more than 2000 products in the peptidome, of which only a quarter are actually derived from the salivary glands indicating that other cells in the oral cavity (e.g. epithelial cells, leukocytes, etc.) make a significant quantitative contribution of proteins to saliva (1). Although salivary secretions are primarily associated with maintaining a moist mouth and digestion of starches (amylase) and lipids (lingual lipase), the hormones in saliva are important for defence and repair of the teeth and the soft tissues of the oral cavity and upper alimentary tract, the oral immune response, inhibiting or activating oral tumours, and for some animals acting as pheromones in territorial delineation, mating behavior and maternal nursing (2).

In laboratory rats and mice, submandibular glands clearly exhibit regulatory functions on other body functions, organs and glands consequent to their secretion into the oral cavity (exocrine secretion) and the blood (endocrine secretion). Numerous biologically active polypeptides are involved in growth and differentiation, enzymatic control, homeostatic regulation, and adaptation to stress (3-5). Dysfunctional salivary glands may facilitate the development of the pathologies associated with various disorders, such as diabetes mellitus, sensitivity to toxins (6), and possibly metabolic syndrome. The endocrine secretions participate in the maintenance of the integrity of eosphageal and gastrointestinal mucosa (7), promote hepatic regeneration (8), are essential for maintenance of the reproductive system (9), influence tumour growth (10,11), modulate the severity of systemic inflammatory responses (12), and modulates the responses of adipocytes (13) to catecholeamines. Many of these biologically active polypeptides are growth factors that are synthesized and secreted from the salivary (submandibular) glands as well as from adipose tissue (Table 1). Although many adipokines are found in saliva to date only a few have been shown to be synthesized and secreted from the salivary glands – leptin (14), interleukin-6 (IL-6) (15) and tumor necrosis factor-alpha (TNF-α) (16).

Diseases of human salivary glands arise from a variety of causes such as obstruction (stone formation), infection, inflammation, tumors and autoimmunity (Sjögren's syndrome). These diseases generally result in loss of function with decreased saliva production and an ensuing complaint of xerostomia, which has the following clinical features: (*i*) increased thirst and fluid intake; (*ii*) difficulty in mastication, swallowing, speech and eating of dry food; (*iii*) burning and tingling sensations in the mouth, (*iv*) oral infections, (*v*) abnormal taste in the mouth, and (*vi*) painful salivary gland enlargement. If loss of function is prolonged the incidence of sialadenitis (inflammation of salivary glands), dental caries, periodontal disease and persistent oral infections increases, with an associated decline in the quality of life (31, 32).

Unlike rats and mice few systemic functions have been demonstrably modified by loss of salivary gland function in humans, although mucosal integrity is possibly modified by low

Table 1. Growth factors secreted from submandibular glands and adipose tissue

Growth Factors	Submandibular glands	Adipose tissue
Epidermal growth factor (EGF)	(17)	?
Nerve growth factor (NGF)	(18)	(19)
Transforming growth factor-alpha (TGF-alpha)	(20)	(21)
Transforming growth factor-beta (TGF-beta)	(22)	(21)
Basic fibroblast growth factor (bFGF, FGF2 or FGF-β)	(23)	(24)
Insulin-like growth factors (IGF-I)	(25)	(26)
Brain-derived neurotrophic factor (BDNF)	(27)	(19)
Hepatocyte growth factor (HGF)	(28)	(29)
Vascular endothelial growth factor (VEGF)	(30)	(24)

levels of salivary EGF thus contributing to Barrett's columnar lined oesophagus (33, 34). The increases in salivary chromogranin A (35) and IgA (36) (antibacterial and antimicrobial properties), after upper thoracic massage have been proposed to contribute to enhanced immune defence associated with these therapies in cancer and HIV patients. Several human systemic diseases that affect the oral fluid's composition include cystic fibrosis, multiple sclerosis, graft-versus-host disease, diabetes mellitus, alcoholic liver cirrhosis, acquired human immunodeficiency syndrome, epilepsy, burning mouth syndrome, kidney dysfunction (37) and metabolic syndrome (38). Other causes of insufficient saliva production include anxiety, dehydration, chemo- and radiation therapy, and some medications such as antidepressants, amphetamines and antihistamines.

Salivary glands and obesity

Obesity *per se* does not appear to impact dramatically salivary gland function. In a recent study (39) rats fed a "cafeteria diet" became obese and showed a 20-30% increase in parotid gland weight, although the weights of the submandibular and sublingual glands were not altered. The histological organization of the glands was not altered in obese rats, although morphometric analysis pointed to a reduction in the number of serous acinar cells (protein secreting) in the submandibular glands.

However, parotid gland enlargement is not unique to obesity as female patients with psychosomatic eating disorders, such as anorexia nervosa and bulimia nervosa (Fig. 1), are also enlarged

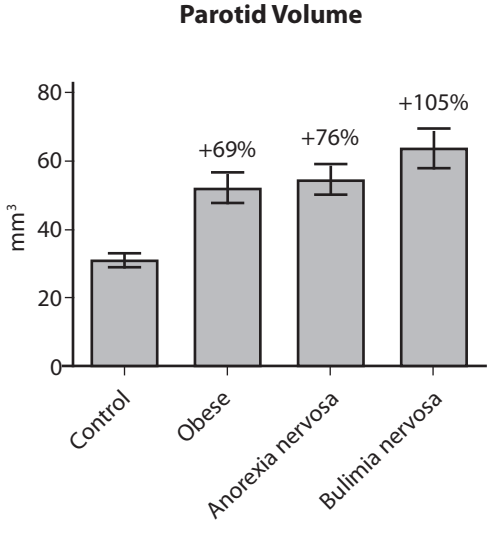

Figure 1. Parotid cell volume is increased in obese patients as well as in non-obese patients with anorexia nervosa and bulimia nervosa. Adapted from (39).

(40). The factors and pathogenesis leading to this enlargement have not been identified for these different eating disorders. The submandibular glands were not affected. The effect of obesity on salivary flow rate is not a settled issue with several groups reporting no effect (41,42), and another suggesting a reduced flow rate (43). Nonetheless, saliva composition changes in directions favouring the formation of caries (e.g. decrease in phosphate and peroxidase activity with increase in free sialic acid and protein (42), thus suggesting detailed studies on saliva composition and the proteome are merited in obese individuals. A recent study examining the proteome showed that obese subjects with periodontitis had higher levels of neutrophil alpha-defensins in their saliva compared to non-obese controls (44). This result is consistent with inflammatory condition associated with obesity. Interestingly, serum alpha-defensins decrease after bariatric surgery-induced weight loss (45), suggesting that these peptides may represent serum markers of inflammation in obese patients (44). Interestingly, a correlation between low plasma level of NGF and reduced excretory function of submandibular glands was found in patients with advanced stage of metabolic syndrome as compared with patients with early stage of metabolic syndrome and with controls (38). This observation in conjunction with the role of submandibular gland EGF decreasing the lipolytic effect of adrenaline (13) suggest that the effects of salivary glands and their secreted growth factors on metabolic syndrome merit further investigation.

Periodontal health and obesity

Since increased prevalence of periodontal disease is a well-known complication of type 2 diabetes mellitus, and as obesity frequently leads toward type 2 diabetes mellitus, the question arises: Do obese patients may have exacerbated periodontal disease?

Both plaque index – reflecting dental plaque, and probing depth – a measurement of periodontal pocket, are closely linked with periodontal inflammation and infection, and are statistically associated with high body mass index (BMI) and obesity, independently of dietary patterns and insulin resistance. Small increases in probing depth values in obese patients possibly indicate an impaired immune response and predisposition to periodontitis (46,47). In addition, patients who have undergone bariatric surgery showed an improved probing depth and clinical attachment level response to non-surgical periodontal therapy when compared to those did not have such a surgery (48). Several meta-analyses have shown a consistent positive association between periodontal disease and BMI, although the magnitude of the association needs to be quantified, temporal associations clarified and mechanisms defined (49, 50). It remains unclear

whether obesity is a risk factor for periodontal disease of whether periodontitis possibly increases the risk of weight gain (49).

Metabolic polypeptides in saliva

Numerous metabolic polypeptides are present in saliva and are synthesized locally and/or transported from the blood. Receptors for most of these polypeptides are expressed in taste cells (TCs): insulin (51), leptin (52), glucagon (53), glucagon-like peptide-1 (GLP-1) (54), ghrelin (55), galanin (56), cholecystokinin (CCK) (57), vasoactive polypeptide (VIP) (58, 59), neuropeptide tyrosine (NPY) (60) and polypeptide Y (PYY(3-36) (61). Receptors for adiponectin have not been described in taste buds but they are present in gingival tissues (62). For the most part these polypeptides and their receptors are linked to putative roles in the different tastes – sweet (52-54, 59), salty (55), sour (54, 55) and umami (63). However, it is only recently that a role for one of these peptides in regulating feeding behaviour was elucidated (61).

Peptide YY(3-36) is a satiation hormone released postprandially into the bloodstream from L-endocrine cells in the gut epithelia, and has been associated with conditioned taste aversion (64). Salivary PYY(3-36) originates from the blood and is also synthesized in cells of the taste buds, and its receptor, Y2R, is expressed in the basal layer of the progenitor cells of the tongue epithelia and von Ebner's gland, which are serous salivary exocrine glands residing within the moats surrounding the circumvallate papillae in the posterior one-third of the tongue. Increasing salivary PYY(3-36), whether acutely with an oral spray or in a sustained manner using viral vector-mediated gene delivery to salivary glands, alters feeding behavior (61). Oral administered peptide induced stronger satiation, and the long-term increase mediated with the viral vector reduced food intake and body weight in diet-induced obese mice. This study is significant on several fronts: (*i*) the view that PPY(3-36) is thought to contribute to conditioned taste aversion due to its ability to inhibits feeding of rodents may actually be a satiation response, (*ii*) a potentially simple and efficient therapeutic approach for the treatment of obesity may be developed, and (*iii*) feeding studies with other metabolic polypeptides may have to be re-evaluated.

Saliva as a diagnostic tool: an adipokine insight

The argument is frequently made that since saliva is an easy to obtain biological fluid and can be collected non-invasively, measurements of salivary hormonal changes should be preferred in diagnoses and treatments. Saliva collection would not burden healthcare with additional costs, and it can be collected readily by patients at home and at various times of the day. However, saliva is not a mainstream sample source for hormone analysis, although for specific investigations in psychiatry, stress research, and pharmacokinetics, saliva analysis delivers very reliable results (65).

For the adipokines the claim is also made that determination of their levels in saliva would contribute significantly to understanding their physiology and role in several clinical conditions such as obesity, insulin resistance, inflammation, reproduction, and stress responses (66). Adiponectin has received the most attention since a significant correlation exists between plasma and salivary adiponectin levels. Since obese, type 2 diabetes and metabolic syndrome patients have decreased levels of plasma adiponectin (67), nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) (68), salivary adiponectin, NGF and BDNF may be a useful measure of increased cardiometaboic risk (69). A significant negative correlation is found between plasma adiponectin levels and BMI (70). However, correlations between plasma and saliva adiponectin levels do not exist with several different methods of collecting adiponectin (70), and a factor exists in saliva that elutes with adiponectin and acts as an inhibitor of adiponectin binding (71). The issues surrounding the measuring of salivary adiponectin are not settled. Thus, there are many encumbrances to making saliva a mainstream sample source for both research and clinical analysis of adipokines. These encumbrances include: (*i*) assay validation in terms of hormone recovery, linearity, accuracy and precision; (*ii*) the need to develop specific and standardized collection methods, and analytical tools; (*iii*) the establishment of defined reference intervals; (*iv*) validation in and implementation of round-robin trials (inter-laboratory testing performed independently several times); (*v*) overcoming the significant issue of patient compliance in issues related to collection times, methods and sample handling, and (*vi*) acceptance over the long term of these methods by clinicians. Even with these cautionary notes future studies may reveal one or more of salivary adipokines (66), proteins (e.g. C-reactive protein) and enzymes (e.g. lysozyme) (72), and/or metabolites (e.g. uric acid) (73) that eventually may be useful for studying, diagnosing and treating metabolic syndrome and obesity.

Treatment of radiation-induced salivary dysfunction

The primary treatments for managing head and neck cancer are radiation therapy or surgery, or both combined. Chemotherapy is used as an additional, or adjuvant, treatment. The side effects of radiation therapy include sore throat, loss of taste sensation and dryness of the mouth, with the latter effects resulting from radiation-induced salivary dysfunction can be particularly severe and problematic in the long term. To minimize these salivary gland problems the clinical focus has been on intensity-

modulated radiation therapy (IMRT) over two-dimensional external beam radiotherapy (EBRT) as a means to spare the major salivary glands and prevent intractable hyposalivation. Several clinical studies have shown that IMRT of oropharyngeal and hypopharyngeal cancers results in a significant reduction in grade 2 xerostomia (moderate dryness of the mouth, poor response to stimulation) over patients receiving standard EBRT (see (74)). Although these results are promising alternative treatment strategies for xerostomia are required since IMRT does not totally spare the salivary glands and is not always available as a treatment option. Furthermore, many of the more than half-million patients treated each year with head and neck cancer will suffer from radiation-induced salivary gland dysfunction consequent to a reduction in function and number of acinar cells and morphological deterioration consequent to inflammation and fibrosis (75,76). Various pharmaceutical treatment strategies (adrenergic and muscarinic receptor agonists) (75) and basic fibroblast growth factor (77) can reduce radiation-induced xerostomia, but these treatments cannot address the patients already suffering irreversible hyposalivation.

Based on advances in using adipose stem cells in a wide variety of therapeutic conditions (78), including their neuroregenerative potential (79), these stem cells were given to mice with radiation-induced xerostomia (80). Adipose-derived stromal cells (ADSC) significantly improved salivary flow rate in mice when implanted into submandibular glands as late as 10 weeks after 10 Gy irradiation treatments (Fig. 2). Some significant features of this ADSC treatment are: (*i*) it occurs rapidly, within 1 month of transplantation, (*ii*) the ADSC differentiate into blood endothelial cells and ductal cells, and (*iii*) the ADSC appear to act by improving blood flow, probably consequent to the strong angiogenic capabilities of these cells with their expression and release of growth factors, such as VEGF and HGF. Some outstanding issues that remain to be resolved are: (*i*) why is there an increase in saliva flow given that ADSC did not differentiate into acinar cells, and (*ii*) the possibility of tumor growth with this stem cell therapy.

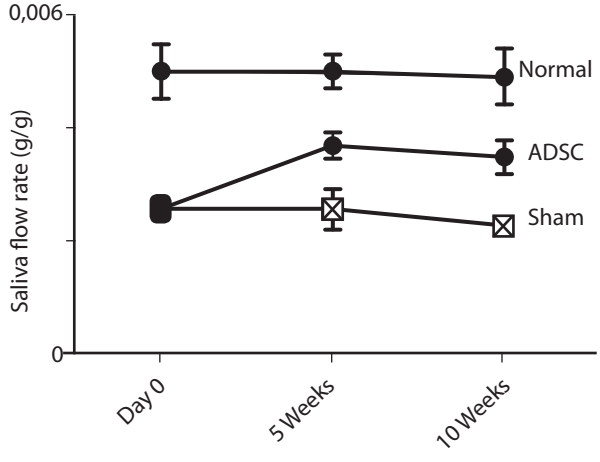

Figure 2. Saliva flow from irradiated submandibular glands is partially restored by transplantation of adipose derived stem cells (ADSC) into the glands 10 weeks after irradiation. Saliva flow rate was measured for 30 minutes after pilocarpine injection on day 0, and at 5, and 10 weeks. Adapted and modified from (80).

This technological approach is significant as it provides a means to improve severe intractable salivary gland dysfunction and can be administered shortly or years after the radiation-induced damage has occurred.

Conclusion

The salivary glands and their "classical" functions – protecting the oral cavity, maintaining a moist mouth, and aiding in digestion – do not appear to be seriously compromised by obesity, although obese patients would be advised to pay attention to their oral health due to a higher incidence of periodontal disease. Sialoadenectomy has been a useful tool for revealing the role of salivary glands in systemic health (81,82), and such a "knockout" experimental approach has not yet been applied to studying the roles of the salivary glands on the development of obesity and metabolic syndrome. A true understanding of salivary gland function in obesity and its related diseases lies in the future if indeed factors released from these glands participate in the satiety and inflammatory responses, offering an avenue for the treatment of these diseases. From the opposite direction adipose tissue may provide therapeutic benefit to dysfunctional salivary glands as transplantation of ADSC may eventually be useful for the treatment of intractable irradiation-induced xerostomia. Last not least, (*i*) the introduction of salivary determinations of adipokines could contribute to the study of pathogenesis of various obesity-related diseases, and (*ii*) the role of adipose tissue associated with salivary glands should also be studied.

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