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**Promoting Wellbeing:
Amylase as an Indicator of Changes in Stress Level in People with
Intellectual Disabilities**

Nigel A. MARSHALL ¹⁾ Kagari SHIBAZAKI ²⁾

1) School of Education and Social Work, University of Sussex. Falmer, UK

2) Department of Psychology, University of Huddersfield, UK

ABSTRACT

In this paper we present the results of two small scale, pilot studies which explore the use of a small hand-held monitor used for measuring the relative levels of the digestive enzyme, Amylase, in the saliva of a population of adolescent children with intellectual disabilities, who experienced a music concert. Our hypothesis was that experiencing the concert would significantly reduce the levels of stress in each individual and thus promote an increased level of wellbeing. The study also focussed on exploring the extent to which salivary amylase activity (SAA) can be measured and used as an indicator of relative changes in levels of stress and ultimately, to see the extent to which such measurements could give 'voice' to individuals with intellectual disability. In the event, our hypothesis was not supported in that participants did not display decreased levels of SAA to a level of significance. However, further analysis and triangulation of the initial results through the case notes of each individual suggested that in fact the SAA measures were accurate and that the expectation that all participants would respond in an identical fashion, had been un-realistic.

<Key-words>

salivary biomarkers, amylase, stress, wellbeing, intellectual disability

n.a.marshall@sussex.ac.uk (Nigel A. MARSHALL; UK)

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I. Background

Stress can impact in a negative way on the overall health and wellbeing of an individual (McEwan, 2008). Authors such as Selye (1978) have described stress as the 'wear and tear' which impacts on the body with numerous studies highlighting significant links between high levels of stress and for example, cardiovascular disease (e.g. Rosmond & Bjorntorp, 2000). Some authors have even gone so far as suggest that stress, and the way the body responds to it, are the two major factors which can negatively impact on health and well-being – even amongst young people (Roemmich, Lobarinas, Joseph et al., 2009; Roemmich, Lambiase, Balantekin et al., 2014). In addition, a wide range of studies have indicated how stress levels appear to impact significantly on a number of both physical and mental conditions. Physical impacts can include accelerating the ageing process (Hawkley & Cacioppo, 2004; Vitlic, Lord & Philips, 2014); the time required for tissue to heal (Kiecolt-Glaser, Marucha, Malarkey et al., 1995) and increased susceptibility to infection as a result of a decrease in the immune system (Glaser & Kiecolt-Glaser, 2005; Vitlic, Lord & Philips, 2014). Mental impacts can include loss of motivation, lack of energy and reduced activity levels (Stults-Kolehmainen & Sinha., 2014; McArdle & Jackson, 2000), eating disorders (Het, Vocks, Wolf et al., 2015; Trottier, Wonderlich, Monson et al., 2016) and sleep quality (Kim, Chang, Hong et al., 2015; Lattova, Keckeis, Maurovich-Horvant et al., 2011).

Stress is usually recognised as incorporating two components. First, when the individual perceives what they consider to be a threat or danger and second, when the body responds to that perceived threat. Bodily responses have traditionally been categorised as 'fight or flight'; that is when the body prepares to either run from the perceived threat or to stay and confront it. Any perceived threat or fear (rational or irrational) activates the hypothalamic-pituitary-adrenocortical axis (HPA) resulting in a significant increase in the level of hormones being released into the blood stream; including cortisol, amylase and adrenaline. The effect of this increase in hormonal activity leads to an increase in heart rate and blood circulation and enables sugar and fat to be processed rapidly in case additional energy is required (Nader, Chrousos, & Kino, 2010). In this respect, physiological changes can be a more accurate measure of bodily responses to stress.

The ability to talk about changes in our emotions and to explain and discuss the relative levels of stress and wellbeing we sense, is a difficult task for anybody. However, for an individual with a severe intellectual and / or physical disability, this can be very difficult. Problems with limited levels of language and vocabulary can be a major hurdle in explaining feelings, stresses and emotions and for individuals with additional issues, such as sight loss, the codes and practices associated with facial expressions and other

social codes are not always available. Therefore as Yamaguchi, Takeda, Onishi et al. (2006) point out, any *'communication system for children and adults with intellectual disabilities (ID) is a desirable assistive technology'* (p.30).

Research into relative stress levels has often employed observations, rating scales and self-evaluation measures in order to investigate the level of stress experienced through exposure to a perceived threatening event. However, Monroe (2008) has pointed out that even amongst the most frequently tried and tested self reported measures (see for example Brown and Harris, 1978), the chances of obtaining reliable data; *"is generously, at best, even odds"* (p.39). Therefore, the identification and use of biomarkers that respond to, and provide reasonably accurate, quantitative measures of changing stress levels are of value not only to future research but also to those involved in a wide range of health care contexts, (Robles, Shetty, Zigler et al., 2011).

Traditionally, cortisol has been used a useful biomarker in assessing relative levels of stress in individuals (Hellhammer, Wüst & Kudielka, 2009; Khalfa, Bella, Roy et al., 2003; Kirschbaum & Hellhammer, 1994; Kreutz, Bongard, Rohrman et al., 2004). However, the relatively slow reaction time of cortisol to stressful events and the naturally occurring fluctuation of cortisol levels throughout the day create a number of additional methodological issues. As a result, more recent studies have demonstrated that increased levels of Salivary Amylase Activity (SAA) correlates equally well with both physical and mental induced stress events (Chatterton, Vogelsong, Lu et al., 1996; Ieda, Miyaoka, Kawano et al., 2012; Nater, Rohleder, Gaab et al., 2005; Natter, La Marca, Floin et al., 2006; Nater, Abbruzzese, Krebs et al., 2006; Nater, Rahleder, Schlotz et al., 2007; Nater & Rohleder, 2009; Rohleder, Nater, Wolf et al., 2004; Rohleder & Nater, 2009; Skosnik, Chatterton, Swisher et al., 2000). An additional benefit to using SAA arises from the fact that relative changes in levels of SAA can be obtained on a regular basis as the response to stress time is far less than with cortisol. Thus for example, a number of recent studies have included numerous SAA measures to be taken with very short intervals in between each individual measure (Arai, Sakakibara, Ito et al., 2008; Kato, Ohinoya, Hasegawa et al., 2011; Shimazaki, Matsuki, Yamauchi et al., 2008).

However, studies involving collecting and analysing either cortisol or amylase have tended to require additional resources, including laboratory equipment appropriate for carrying out the analysis. The analysis of the salivary amylase samples in our current study however, involved the use of a hand-held SAA monitor developed by Nipro Co. Japan (see Higashi, Mizuno & Yamaguchi, 2005; Robles, Shetty, Zigler et al., 2011; Shetty, Zigler, Robles et al., 2010; Yamaguchi, Kanemori, Kanemaru et al., 2003; 2004; Yamaguchi, Deguchi, Wakasugi et al., 2006). Salivary amylase was collected in a pre-post test design using a disposable collector strip (Nipro Co. Japan). Analysis of the sample

was carried out immediately and in-situ using the hand held reader. Given that the participants included in the current studies had all been registered as having an intellectual disability, the research options available to the research team were limited. For example, questionnaire or interview or any tool requiring reasonable levels of comprehension or language and vocabulary were not appropriate – given the relative low levels of communication skill in our current research population. In this respect, obtaining and using changes in levels of amylase as an indicator of change in the level of stress (increase or decrease) experienced by each participant, was highly appropriate.

Previous studies have highlighted the positive effect which music can have on our emotions (Juslin & Sloboda, 2010; Kreutz, Bongard, Rohrman et al., 2004; McKinney, Antoni, Kumar et al., 1997; Mockel, Rucker, Stork et al., 1994; Nakayama, Kikuta & Takeda, 2007). However, simply trying to investigate the preferences, tolerances and likes/dislikes which individuals may possess is a highly contentious and problematic area of study. Although we all assume we have musical preferences, these are in fact, far from stable entities and our preference for, tolerance of and like /dislike for certain musical pieces can be continuously affected by, for example, the physical location in which we hear the music (Martindale, 2007, 1990; Martindale, Locher & Petrov, 2007; Martindale & Moore, 1988); the complexity of the musical pieces (Heyduk, 1975), along with a whole range of other factors (Han, 2003). Such issues are further complicated amongst individuals with little or no communication skill. However, a range of previous studies have highlighted the effectiveness of using salivary biomarkers in order to assess the relative impact of musical experiences (Bartlett, Kaufman & Smeltekop, 1993; Edwards, Clow, Evans et al., 2001; Tsao, Gordon, Maranto et al., 1991), and therefore, in this paper, we present the initial findings from two pilot studies.

Study number one explored the use of the salivary biomarker *amylase*, both as an indicator of stress and wellbeing in adolescents with severe intellectual disabilities but the study also enabled the piloting of the hand-held SAA monitor produced by the Nipro Co. Japan. Study number two employed a post hoc analysis of case notes carried out by the key workers for each individual participant. This second study was carried out in order to enable us to triangulate and augment data from study one and to further explore any anomalies arising from the results of first study.

The beneficial effect of music on reducing levels of stress is well documented (e.g. Thoma, La Marca, Bronnimann et al., 2013). Thus, given the fact that previous studies had identified the positive impact which musical experiences can have in reducing levels of stress, our hypothesis was that the levels of SAA in participants would reduce significantly as a result of partaking in a one hour musical event.

Therefore, the two research questions of the study were as follows:

- 1) To explore any relative changes in levels of SAA amongst young participants with intellectual disabilities in response to a live musical event.
- 2) To explore the use of the hand-held amylase monitor as a means of measuring stress levels in participants with intellectual disabilities.

Ethical permission for the research was given by the UK university. As all participants were aged under 18 years old, permission was initially sought and gained from parents. However, even where parental permission was obtained the final consent was left to each individual. Any participants who did not wish to take part at any point, was omitted from the sample.

II. Method - Study One

Our procedure involved collecting 102 measures of SAA, in a pre-post test design. All participants were adolescents enrolled in a state-run special school specifically catering for children with intellectual disabilities. Two professional musicians performed in the communal setting within the school. A small saliva sample was obtained from each participant both before and after a one hour music concert. Samples were collected using the designated spatula designed by Nipro Co. Japan, for use with the hand-held monitor. Samples were collected by a member of the school staff who was known to the participant and all saliva samples were analysed immediately. In order to prevent any emerging bias, the collection of the SAA was carried out under 'blind conditions' – that is, the individual with responsibility for the saliva collection was trained in using the monitor and in recording the results, but they were not informed as to whether or not the results they obtained were positive or negative.

The intervention took place during the afternoon school session and lasted for one hour. The timing of the event was crucial and took place between 1.30 and 2.30pm. Amylase is essentially a digestive enzyme and therefore increased levels of amylase are produced before and during meals for example. Subsequently, in addition to responding to stress, increased levels of SAA can be provoked by an expectation that food will soon be forthcoming e.g. when approaching a meal time; or the smell of food preparation throughout the institution, or any food or drink, other than water, consumed in between the pre-post event measure (see Rohleder & Nater, 2009). Therefore, the period between 1.30 and 2.30pm was critical in that this provided a window of opportunity between fixed meal times, with all on site food preparation having finished. In total, 102 SAA measures were taken; 51 pre-event measures and 51 post- event measures.

III. Results – Study One

Our hypothesis argued that the levels of SAA in each of the participants would decrease significantly as a result of experiencing a one hour musical event. All samples were analysed immediately using the hand held SAA monitor and readings recorded in SPSS. Since amylase activity varies significantly between individuals (e.g. as a result of medication or individual physical conditions), all data levels were converted into logarithmic values using $\log(x+1)$ in order to transform the data (see Robles et al., 2011). Overall, our hypothesis was not supported. Of the 51 cases, 32 (62.7%) did report the expected decrease in levels of SAA but the remaining 19 cases (37.3%) reported an unexpected increase. A paired-sample t-test was conducted in order to evaluate the impact of the intervention on levels of SAA and this suggested whilst there was an overall decrease in the means of SAA [Pre test: (M=54.23, SD=34.66); Post test: (M=46.57, SD=32.03)]; statistically this did not reach a level of significance ($t(50) = 1.41, p=.164$ (two tailed)).

IV. Considerations and Conclusions – Study One

In terms of the first research question, the pre-post test design did provide an indication of changes in levels of SAA amongst participants. However, results suggested that whilst there was a general trend towards supporting our hypothesis, this did not reach any level of significance with almost 40% of participants showing increased levels of stress – as indicated through an increase in their levels of SAA. There could be a number of reasons for this, but the most likely would be, first, that some error occurred during the saliva collection process. For example, a slight delay occurring between the time of the collection and the time of the analysis. Second, there could be an issue of reliability and validity with the hand held monitor and third, it could be that experiencing the live musical events, or some associated factor did in fact bring about an increase in the levels of stress experienced by the individual participants.

In consideration of the above, first we accept that an error in the collection of the saliva sample is always a possibility although the research team did provide training in this technique. Second, although it is possible that the hand-held monitor was prone to a lack of reliability and validity, the extensive research done in development would argue against this (see for example Higashi, Mizuno & Yamaguchi, 2005; Shetty, 2010; Yamaguchi, Kanemori, Kanemaru et al., 2003; 2004; Yamaguchi, Deguchi, Wakasugi et al., 2006). However, it is possible that some aspect of the music concert, or a related aspect of attending the concert, did create a genuine increase in the stress level

experienced by a particular individual. If this were found to be true, this would indicate that one advantage of this simple measure of SAA could be seen as giving voice to individuals to whom other forms of communication are not necessarily possible.

In terms of our second research question, the collection of SAA was carried out quickly and in a painless and non-intrusive way by a known member of staff. Analysis was carried out quickly and all samples were disposed of immediately following the standard procedures associated with the disposal of human tissue. No adverse effects were reported with the use of the monitor and analysis of the data suggested that salivary amylase was a sufficiently sensitive measure for exposing sometimes relative minor changes in stress levels over a limited period of time. However, although the mean values of our pre-post test data suggested that overall, the musical events did promote a decrease in levels of stress, as indicated by levels of SAA, we were unable to further explain the mixed results in terms of the increase SAA in 19 participants. Accordingly, we therefore posed one further research question in order to explore the context in which the musical event took place, and to establish if some external variable could account for the increase in SAA in 19 of the participants, namely:

- To what extent can additional, contextual factors account for the changes in SAA experienced by participants ?

Our focus here was to try to establish the extent to which the level of SAA measure, as recorded by the hand held monitor was accurate by comparing the SAA reading with case notes made by staff members in which experiences, other than the musical ones, could be exerting a level of influence. In this respect, the overall research design can be explained as '*sequential explanatory*' (Creswell, 2008). That is, a sequential design in which anomalies and unexpected results from an initial data analysis are more specifically explored and 'explained' through a more qualitative approach.

V. Method – Study Two

The data used in our second study consisted of an analysis of the case notes of each individual. Case notes on individual participants were prepared by all staff members as part of their normal professional duties and all personal or identifiable information was removed prior to analysis by the research team. However, individual identities were coded enabling a match to be made between the results of the SAA sample and the case notes for each individual. Summative sheets were prepared which detailed the main positive or negatives events & experiences of the day alongside the recorded pre-post test amylase measures. This enabled a clear comparison to be made between the result of the SAA sampling (increase or decrease) and any positive / negative factors reported in the

case notes of the individual. In each individual case, we identified instances where SAA had increased and cross referenced this with the corresponding case notes in order to identify any correlation between the increase and the noted events / experiences within the case notes. A similar task was then carried out on those instances where the SAA levels had decreased.

VI. Results – Study Two

Comparing the results of the SAA measure and the individual participant case notes enabled a more detailed account to be made of the individual participant experiences and enabled us to set the SAA measures into an overall context. Case notes were carried out as part of normal professional practice by key workers who were not aware of the purpose of the study. In this respect, key workers were not clear as to how comments could, or would add support to our hypothesis and therefore this limited the opportunities for key worker bias.

Participants with decreased levels

We accessed the case notes of a random sample of 10 (out of 32) case notes on participants who had recorded a decrease in their levels of SAA. In each case, there was evidence of situations in which the individual was affected by the music in a positive way. Comments which reported participants displaying increased levels of cooperation, positive changes in mood, smiling and making other expressions of happiness and enjoyment frequently appeared in instances where the levels of SAA were seen to decrease. For example,

<Table 1> Participant 5

Case Note comment Pre event	Case Note comment Post event	SAA – Pre event	SAA – Post event	Difference
Difficult morning–not happy to be at school. Kept moving table. Refused to work. Appeared sad.	Much more settled	173	58	Decrease of 115

<Table 2> Participant 2

Case Note comment Pre event	Case Note comment Post event	SAA – Pre event	SAA – Post event	Difference
Upset after witnessing a lunchtime incident	Calm and happy	119	33	Decrease of 86

In all 10 cases, comments made by key workers in case notes supported the decrease in levels of SAA within each individual. That is, had case notes been seen prior to the measure of SAA, we would have predicted the decrease.

Participants with increased levels

Of the 19 cases in which participants displayed an increase in SAA, a random sample of 10 case notes were again selected. In 7 of the cases, the detailed case notes supported (i.e. could have predicted) the idea that an increase in levels of stress had taken place as a result of one aspect of the concert, or an experience taking place within the concert; suggesting that the SAA measure had in fact been accurate. The first common cause for the increase in stress related to the volume of the music. This was a feature that appeared in a number of cases, and proved to be an issue even amongst some of those who responded to the concerts with decreased stress levels. In the second category, the increase in stress appeared to be the result of some external event which either combined with the volume of the music or contributed to the level of stress.

<Table 3> Participant 4

Case Note comment Pre event	Case Note comment Post event	SAA – Pre event	SAA – Post event	Difference
Very happy – talking about his news, keen to go to the concert – looking forward to the music.	Left the hall upset during the concert due to the noise being too loud. 5 minutes outside and then returned	26	52	Increase of 26

<Table 4> Participant 7

Case Note comment Pre event	Case Note comment Post event	SAA – Pre event	SAA – Post event	Difference
Anxious about annual review meeting. Keeps asking about it. Went to concert.	Didn't enjoy a lot – could not understand the concert as much as the last one.	24	39	Increase of 15

In three instances, no issues were reported which could explain an increase in stress and in each case, participants were not able to communicate a reason verbally. Case notes in these instances tended to focus on more clinical matters.

Overall, and in all but three instances, the increase or decrease in levels of SAA were supported by the wider context recorded within the case notes giving an indication that the actual SAA measure was generally, an accurate one. Further participant 'preferences' – as evidenced by increased levels of activity, were expressed for music or songs with which participants were familiar, rather than non-familiar musical items and in addition, we also noted that a number of the participants found the 'noise' rather than the 'music'

to be too loud. That is, the response of the other audience members was the cause of the increased stress, rather than the actual volume of the music itself.

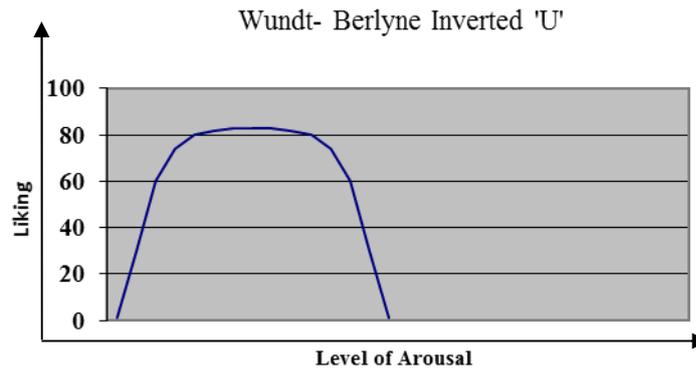
VII. Discussion

Thus, it appears that although the pre-post test results did not produce a significant result and therefore did not support our original hypothesis, it does seem that there is limited evidence to support the notion that the levels of SAA were an accurate measure of the levels of stress in individuals; given that those instances which displayed an increased level of SAA appeared to be accounted for by other external factors and could have been predicted by the case notes. However, one further question to be addressed relates to the fact that whereas some participants did arrive at the event in an already stressful condition, the musical event did have a positive effect and a decrease in SAA was reported (see e.g. participants 2 & 5 above). Conversely, others arrived in a relatively non-stressed condition and found the event to be stressful and in these cases, an increased level of stress was recorded (see participant 4 & 7). Two possible issues could account for this. First, the case notes were subjective and therefore whilst they did actually record incidents and possible indicators of experiences which could promote stress, we have no precise measure of how stressed / aroused the individual actually felt. Second, there is also a further theoretical basis which links levels of stress to our ability to process and enjoy or tolerate music.

In order to understand this further theoretical explanation for the variation in SAA levels, we first need to report briefly on the theory of arousal and liking, as proposed by Wundt (1874) and Berlyne (1971, 1974). Wundt proposed, and Berlyne adapted, an inverted 'U' curve which plots our levels of arousal against our liking or preference for a particular experience. Following Berlyne's (1971, 1974) adapted theory, individuals display a liking / preference for a musical stimuli, that creates the "optimum" level of physiological arousal. The curve predicts that if the arousal level of the music is too low (e.g. slow and quiet), then we perceive the music to be boring and we dislike it. Correspondingly, if the arousal level is too high, for example if the volume of the music is too high or the music too complex, then we are equally likely to reject or dislike it. However, within the reality of everyday life, each of us as individuals, tend not to begin an experience from the lowest point on the curve.

Daily events cause us to move in and out of different levels of arousal and therefore we also need to factor into the inverted 'U', the precise point at which we, as individuals, enter the 'U' curve. For example, if we are already highly aroused after an argument with a colleague, then we enter a particular experience at a higher point on the curve. This

therefore affects the level and amount of additional arousal we are able to psychologically process. That is, according to the Wundt- Berlyne curve, we can process music that is far more complex and arousing when we are relaxed than when we are in a highly aroused, stressed or anxious state (see also Konečni, 1979; 1982; Konečni & Sargent-Pollock, 1976). Thus, entering into an experience from an already existing state of high arousal can impact significantly on our preference / liking for that experience.



<Figure 1> Wundt/Berlyne Inverted 'U' of Liking against Arousal (1971)

Based on this theoretical idea, we hereby suggest that the levels of stress experienced by each participant, could in fact be relatively, accurately reflected in the measures of SAA as measured by the hand-held monitor. That is, the musical event – or circumstances surrounding the context of the event, could act on the individual to reduce stress levels but also, depending on the level of arousal prior to the concert and / or the potential levels of arousal within the experience (e.g. volume, known/ unknown music, noise of the audience etc.), act to increase their apparent stress levels.

VIII. Conclusions

Therefore, we suggest that some aspects of musical experiences, or the public contexts in which they take place, can in some instances promote increased levels of stress, in the same way as they can also act positively and decrease levels of stress. In our study, some individuals were able to control this effect either through the use of additional aids such as ear defenders, or by removing themselves from the experience and returning as and when they wished to do so. By engaging in daily life, we are all subject to numerous, varied experiences and events which interact with, and impact on the level of arousal our bodies experience. Naturally, it appears that the individuals in our sample also responded effectively and normally to a plethora of additional experiences which impacted on their ability to process the musical material and in this respect, our

hypothesis was unrealistic. In the same way that we, as individuals do not respond to our favourite piece of music in exactly the same way each time we hear it, our participants were also subject to the daily issues and impacts which engaging in society brings.

Similarly, we suggest that the information provided through the measurement of SAA can be, as Yamaguchi, Takeda, Onishi et al. (2006) stated, an effective '*communication system for children and adults with intellectual disabilities (ID)*' (p.30), in that it appears that in this relatively small sample, the levels of SAA were accurately measured – as supported to some extent by the case notes provided by key workers. If further work on a larger and broader population could be carried out, then this could contribute to our better understanding of the behaviours exhibited by individuals with little or no other means of communication. Certainly, there are many individuals living with far more complex disabilities and needs who are realistically unable to accurately express, convey or communicate their preferences, and their likes and dislikes. Those living with final stage or severe dementia for example, or those individuals living with profound, multiple learning difficulties (PMLD), could certainly benefit from an additional 'communication system' in order that those who care for them can be more effective in reducing their levels of stress and increasing their overall level of wellbeing.

In conclusion, we fully acknowledge all the limitations of this pilot study, but we suggest that the initial, and tentative results support the use of SAA as an indicator of the apparent levels of stress that individuals experience. We also suggest that this non-invasive, inexpensive, simple and ethical method provides an opportunity for all those involved in care contexts, to communicate more directly with the individuals they care for and to better understand the effect that a wide range of experiences may or may not have on the individual's physiology and overall wellbeing. This additional level of communication is of further specific importance when dealing with individuals who for a variety of reasons, cannot enjoy the benefit of directly communicating their likes and dislikes to those involved in their care. Recently, a number of authors have both argued and advocated for a more inclusive and emancipatory approach towards carrying out research with individuals who live with intellectual and physical disabilities (Cook & Inglis, 2009; Frankena, 2015; Bigby, Frawley & Ramcharan, 2014; Walmsley, 2001). In short, we argue that the use of SAA as indicator of stress can give a voice to those who otherwise have none; by allowing the physiology to speak for the individual.

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Faculty of Education, University of the Ryukyus, 1 Senbaru, Nishihara, Nakagami, Okinawa, Japan
FAX: +81-098-895-8420 E-mail: ash201091@gmail.com

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Faculty of Education, University of the Ryukyus, 1 Senbaru, Nishihara, Nakagami, Okinawa, Japan
FAX: +81-098-895-8420 E-mail: ash201091@gmail.com

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CONTENTS

ORIGINAL ARTICLES

Associations among the Nutritional Status and Mental/Physical Functions of Care-dependent Individuals Living in Residential Homes for the Elderly

Yuko FUJIO, et al. 1

A Survey on International Nursing Education of National Universities in Japan

Akina ISHIBASHI, et al. 8

Academic Qualification and Job Market Placement: A Bangladesh Study

Rajib Ahmed FAISAL, 17

The Influence of Living Environment on Independence level of Special Nursing Home

Yukie ENOMOTO, et al. 30

Encouraging Exercise Participation amongst UK South Asians:
The Case of a Community Gym

Nigel KING, et al. 46

Promoting Wellbeing:
Amylase as an Indication of Changes in Stress level in People with Intellectual Disabilities

Nigel A. MARSHALL, et al. 60

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