#### Accuracy of GoBe2<sup>™</sup> Smartband in Estimating the Calorie Intake of Food

S. Schaefer, PhD, Foods for Health Institute

December 8, 2018

#### Abstract

The GoBe2<sup>™</sup> is a health and wellness technology with capability to automatically track calorie intake. The wristband gathers information by analyzing the behavior pattern of extracellular and intracellular fluid balance, and predicting the influx of glucose and essential nutrients into the blood in order to estimate calorie intake throughout the day. A study was conducted to test the accuracy of the GoBe2<sup>™</sup> for measuring calorie intake against a direct observation reference method. Food and nutrient intake was measured in 27 healthy volunteers over 14 days. Normality tests and correlation analyses were used to examine the anthropological characteristics of the study sample at baseline. Data were grouped into 3-day intervals to estimate mean intake (kcal/day). Paired sample t-tests compared the mean calorie intake (kcal/day) estimated by the GoBe2<sup>™</sup> and the reference method (d<200 kcal/day), and Spearman's rank order test showed good correlations between the two methods ( $r_S$ =0.70) and strong correlation for all day period ( $r_S$ =0.88). The difference between estimation of the mean calorie intake intake by GoBe2<sup>™</sup> and reference method was 10% (RD; CI: 7%-13%). In conclusion, calorie intake measurement with GoBe2<sup>™</sup> technology provided good correlations to direct observation of food intake in healthy adults.

### INTRODUCTION

Maintaining balance between energy intake and energy expenditure is an important component of achieving a healthy metabolism and body weight over time. Health measurement technologies utilize various methods to estimate physical activity, fitness and energy expenditure, whether through pedometry, accelerometry, measures of heart rate, etc. Yet, estimating calorie intake remains a challenge in the field of wearable sensors.

#### What is GoBe2™?

GoBe2<sup>™</sup> is the world's first and only smartband technology with unique features such as 100% automatic tracking of calorie intake, water balance and stress. GoBe2<sup>™</sup> only needs to be in contact with the user's skin to work, there is no need to enter any data manually into an app.

The band has four sensors (a bioimpedance sensor, accelerometer, piezo sensor and galvanic skin response sensor), which require additional power. The compact built-in rechargeable battery provides a capacity of 370 mAh.

### Accuracy of GoBe2<sup>™</sup> Smartband in Estimating the Calorie Intake of Food

S. Schaefer, PhD, Foods for Health Institute

December 8, 2018

Sensors	GoBe2™ Functions
Piezo sensor Impedance sensor 9-axis accelerometer Gyroscope Magnetometer Galvanic skin response sensor	Calorie intake Calories burned Steps and distance Energy balance Water balance Heart rate Sleep quality Stress patterns Moments of emotional tension

#### Calorie Intake

GoBe2<sup>™</sup> ensures automatic tracking of calorie intake. Using FLOW<sup>™</sup> technology and a bioimpedance sensor, GoBe2<sup>™</sup> assesses the behavior pattern of extracellular and intracellular fluid. This is associated with the influx of glucose and essential nutrients into the blood throughout the day.

### How does GoBe2<sup>™</sup> work?

1. You eat or drink.

2. The food reaches the intestines, where enzymes begin to break it down. The rate of breakdown depends on the contents of the meal and your metabolism.

3. In the intestines, the body breaks food down into glucose to be absorbed by the body. When glucose is absorbed, its concentration begins to increase in the blood. The body's cells then remove the glucose from the bloodstream to use it as fuel.

4. GoBe2<sup>™</sup> uses a sensor to measure the behavioral patterns of extracellular and intracellular fluid 24 hours a day, monitoring the changes in blood glucose, and using this information to track calorie intake.



### Accuracy of GoBe2<sup>™</sup> Smartband in Estimating the Calorie Intake of Food

S. Schaefer, PhD, Foods for Health Institute

December 8, 2018

GoBe2<sup>™</sup> tracks calories when they are absorbed by the body as they enter the bloodstream. The speed of displaying calories in the application may be up to 8-12 hours in accordance with the rate of absorption of nutrients. Calories may be absorbed both during the day and at night (if food was consumed in the evening).

The aim of this study was to evaluate the accuracy of Healbe GoBe2<sup>™</sup> in estimating the calorie intake (kcal/day) of healthy U.S. volunteers. The reference method used was direct observation of measured food intake.

### METHODS

#### Subjects

Study volunteers were 27 adults ages 18-40 (11 males, 16 females). All volunteers agreed to use the GoBe2<sup>™</sup> smartband to automatically track all of their food intake throughout the study, and have all of their food intake measured by the research team. Briefly, this would include consuming three calibrated meals everyday at a dining facility and recording all other food intake. All volunteers were university students or employees recruited to complete two 14-day study periods. This paper present the results from the first two-week period.

### Recruitment & Screening

Approval for the research was obtained from the UC Davis Institutional Review Board. Volunteers were recruited from around the UC Davis campus through emails and flyers. Interested participants were screened by phone for inclusionary and exclusionary criteria. Eligible participants were required to be between the ages 18-50 years and meet defined metabolic screening criteria. Exclusionary criteria included having obesity, defined as having a body mass index (BMI) >30 (CDC), and a historical or current diagnosis of chronic disease (including diabetes or prediabetes, cancer, asthma, hypertension, cardiovascular disease, stroke, kidney, thyroid or autoimmune disease, etc.), known food allergies, presence of abnormal or restrictive dietary practices (i.e., vegetarian, ketogenic), pregnancy or lactation, smoking, drug or alcohol addiction, excessive exercise or athletic training, and medications that influence digestion or metabolism. In-person screenings were conducted at the Ragle Human Nutrition Research Center on the UC Davis campus. Those who qualified after the phone screening were invited for in-person screening to provide written informed consent, a fasting blood draw, blood pressure and anthropometric measurements. Urine pregnancy tests were conducted with all female volunteers. For metabolic screening, blood was tested for abnormal complete blood count, comprehensive metabolic profiles, hemoglobin a1c, and erythrocyte sedimentation rate. Blood drawn into EDTA and PST Lithium Heparin blood collection tubes and

#### Accuracy of GoBe2<sup>™</sup> Smartband in Estimating the Calorie Intake of Food

S. Schaefer, PhD, Foods for Health Institute

December 8, 2018

immediately placed on ice. Within 2 hours of collection, blood samples were centrifuged at 1800 X g for 15 min at 4 °C to separate blood from plasma and frozen at -20 °C until lab analyses were performed in-batch. Test were performed according to manufacturer instructions and quality controls by UC Davis Health System Medical Diagnostics (see Appendix).

For anthropometry, a digital scale by Scaletronix was used to weigh participants to the nearest 0.1 kg. A wall stadiometer was used to measure height in centimeters (cm) to the nearest 0.1 cm. Anthropometric measurements were conducted in accordance with methods defined in the Anthropometric Standardization Reference Manual (Lohan, 1988) and by a trained expert from the University of California, Davis. Blood pressure measurement was obtained using a Nellcor Pulse Oximeter with OxiMax Technology from WelchAllyn.

#### **Testing Protocol**

#### Food intake

The daily food and nutrient intake of study volunteers was measured by a direct observation reference method. The method was developed by the principal investigator and a registered dietitian in collaboration with a student dining facility on the UC Davis campus. In coordination with the facility's scheduled menu cycle, the dietitian prepared a project menu for each day of the study. Items were selected for research study breakfast, lunch, and dinner options that provided balanced macronutrients at each meal per USDA MyPlate guidelines (<u>https://www.choosemyplate.gov/</u>).

The research team analyzed each menu item for calorie and macronutrient content. Nutritional information was analyzed and recorded based on product nutritional labels when available, or the USDA Food Composition database (https://ndb.nal.usda.gov/ndb). The analysis for each menu item followed a similar template, as follows: name, serving size, grams/serving, calories/serving, protein/serving, fat/serving, carbohydrates/serving. Nutritional information for 'standard' servings was scaled to nutrient analysis/100g servings to enable calorie and macronutrient calculations for individual volunteers.

Each day the study menu items were prepared in a commercial kitchen by trained food service personnel following a stringent HACCP protocol. The project dietitian determined 'standard' serving sizes for each menu item (1 c. cooked oats, 1 c. vegetable, ½ c beans, 4 oz. lean protein, etc.) All food was received by a trained team for portioning menu items and serving. Study volunteers were instructed to attend each meal and arrive at the dining facility during a scheduled window of time. Upon arrival, volunteers were greeted by research staff, presented with the 'standard' menu and could request more or less portions of each item to meet individual dietary needs and preferences. The research team weighed, recorded, portioned, served and recorded plate-waste for all study volunteers

#### Accuracy of GoBe2<sup>™</sup> Smartband in Estimating the Calorie Intake of Food

S. Schaefer, PhD, Foods for Health Institute

#### December 8, 2018

at each on-site meal. All meal time staff were trained by the project dietitian for appropriate foodhandling and safety, food weighing and meal recording duties. Individual menu items were weighed with digital scales before and after the meal; the amount of each item consumed was calculated to the nearest 0.1 g, and entered into a database along with the meal time. Data were checked for accuracy by designated members of research team. Data entry staff were trained by the principal investigator.

Volunteers were instructed to only consume foods provided by the project, either at meals served within the dining facility or from a selection of packaged foods (i.e., protein and snack bars, potato chips, instant soup) provided to volunteers to eat in-between meals. Guidelines were also provided if volunteers needed to consume food outside of what was provided through the project, although this was not encouraged. Guidelines included: 1) Avoiding certain foods where it is difficult to accurately estimate energy intake, such as mixed food dishes, 2) Consuming only packaged food items with

nutrition labels from which information could be sourced. All food packages and labels were turned into research staff to estimate the volunteer's energy and macronutrient intake for that item, 3) Weighing all foods. At the enrollment appointment, volunteers were assigned digital kitchen scales and trained by researchers to use it to weigh individual food items. They were given a study notebook contained daily logs to record information about any foods eaten outside of the facility (weight, time, name of food, etc). A team of data entry staff recorded and analyzed the nutritional intake of all reported foods consumed outside of project meals at the dining facility.

#### GoBe2<sup>™</sup> technology

On the afternoon before Day 1 of the study, volunteers arrived to an enrollment appointment where they were equipped with a fully charged GoBe2<sup>™</sup> unit paired with a Healbe smartphone app. They were instructed to consume a simple dinner that evening, with no food after 9 pm. This strategy was important to minimize errors on Day 1 relating to the digestion of calories from the previous day's intake. Volunteers were trained to use the GoBe2<sup>™</sup>; including how the app works to estimate personal

calorie intake and expenditure, heart rate, hydration, stress level and sleep, both in real time and over the previous seven days. Volunteers were instructed to sync the wrist unit with the app twice a day, in the morning and night. Each day, volunteers were asked to provide screenshots from the Healbe app showing the previous day's energy estimations, including intake, expenditure and balance (Figure 1).

**Figure 1.** Screenshot of the Healbe app's Energy function



#### Accuracy of GoBe2<sup>™</sup> Smartband in Estimating the Calorie Intake of Food

S. Schaefer, PhD, Foods for Health Institute

December 8, 2018

#### Quality Assurance

The GoBe2<sup>™</sup>'s current form factor and battery life capabilities present challenges to its ability to acquire data continuously. Improper position of sensor or loss of battery to the unit result in energy miscalculations. Because GoBe2<sup>™</sup>'s signals are derived from the post-prandial (digestive) activities which last several hours beyond the meal, any lapses in data collection during or in the hours after a meal will result in the underestimation of total calorie intake. To mitigate this challenge, sensor contact was monitored closely on a daily basis by members of the research team. Volunteers were advised to fully charge the device each morning prior to any meal. Upon arriving to the first meal of the day, they were checked by research staff who visually confirmed the smartband was properly positioned with the sensor in good contact with the skin. Additionally, the team used a program called Dietitian Cabinet (<u>https://dietologist.healbe.com/</u>) to monitor volunteer data each day. When synced with the GoBe2<sup>™</sup>, volunteer data from the Healbe apps were accessed allowing the team to identify those showing frequent sensor interruptions and data loss. In such cases, individual solutions were developed to improve data collection, such as adjusting the GoBe2<sup>™</sup>'s position on the arm or tightening the wristband.

#### Compliance

Some of biggest challenges in food and diet tracking studies include compliance and accurate reporting. For this reason, continuous blood glucose was monitored as a measure to confirm reported food intake. The FreeStyle Libre Pro glucose monitoring system (Abbott Diabetes Care, Inc.) was used which consists of a small sensor that attaches to the upper arm. It records blood glucose continuously (every 15 minutes) for 14 days, after which data can be downloaded to a reader and uploaded in both report and raw data formats. Sensors were attached to volunteers at the start of the study, prior to the first meal of the day. Sensors occasionally became detached during the two week study on 8 of 27 volunteers causing data loss or interruption until the sensor could be replaced.

### Statistics/analysis

Prior to analysis, a data cleaning process was carried out. Daily cases were eliminated from analyses based on the following criteria:

 CGM data revealed high spikes in blood glucose but no food intake is recorded. Specifically, if CGM data indicated a glucose change >2 mmol/l per 20 minutes, but there was no food intake indicated in the records, it was assumed there was unreported food intake. A total of 12 volunteer/days were removed from analyses for this reason.

#### Accuracy of GoBe2<sup>™</sup> Smartband in Estimating the Calorie Intake of Food

S. Schaefer, PhD, Foods for Health Institute

December 8, 2018

2. Device "non-use", in which the GoBe2<sup>™</sup> was not used sufficiently during or in the immediate hours after a food intake event (i.e., meal, snack). A total of 25 volunteer/days were removed from analyses for this reason.

Calorie intake (kcal/day) data were transformed into 3-day "rolling" averages in attempt to decrease error from instances where food was digested on a subsequent day from which it was consumed. It is necessary to acknowledge that in reality, digestion does not fall within a 24-hour day schedule. When applied to such, it is reasonable to assume that some energy is derived from food ingested during the previous 24 hours. Data were compiled into three day segments for each volunteer and the daily average calculated. For example, for 6 valid study days, four 3-day rolling averages were created:

- Average for Day 1, 2, 3
- Average for Day 2, 3, 4
- Average for Day 3, 4, 5
- Average for Day 4, 5, 6

An arithmetic mean value calculation method was used to determine the statistical error between the reference and measured values of calorie intake. In each allocated interval, the mean of the reference and measured values were obtained. The statistic error for each interval was considered equal to the difference of reference and measured mean values (*d*), divided by the reference mean value. The average statistic error was considered as arithmetic mean value for all counted errors.

All statistical estimates and tests are bootstrap and Monte Carlo. Analyses were conducted in BoxPlotR (<u>http://shiny.chemgrid.org/boxplotr/</u>), PAST (<u>https://folk.uio.no/ohammer/past/</u>) and Reference Value Advisor (<u>http://www.biostat.envt.fr/reference-value-advisor/</u>). Mean calorie intake for both the GoBe2<sup>™</sup> and reference methods were analyzed using paired sample t-tests.

### RESULTS

31 volunteers were initially enrolled in the 14-day study. Three volunteers withdrew during the first five days of the study due to scheduling conflicts with study requirements, and one volunteer's data was lost from device failure. The final analyses included 27 volunteers. Baseline anthropological characteristics of the sample were analyzed (age, weight, height, BMI, systolic (SBP) and diastolic blood pressure (DBP)); the distributions of key variables are displayed by Box-Whisker plots in Table 1. Normality tests were conducted to examine how well the sample variables modeled a normal distribution (if a sample is normally distributed, the plotted points will fall along a straight line). Because some variables had non-normal distributions (i.e., age and BMI, as indicated by p<0.05), non-parametric tests were used in subsequent analyses.

### Accuracy of GoBe2<sup>™</sup> Smartband in Estimating the Calorie Intake of Food

S. Schaefer, PhD, Foods for Health Institute

December 8, 2018

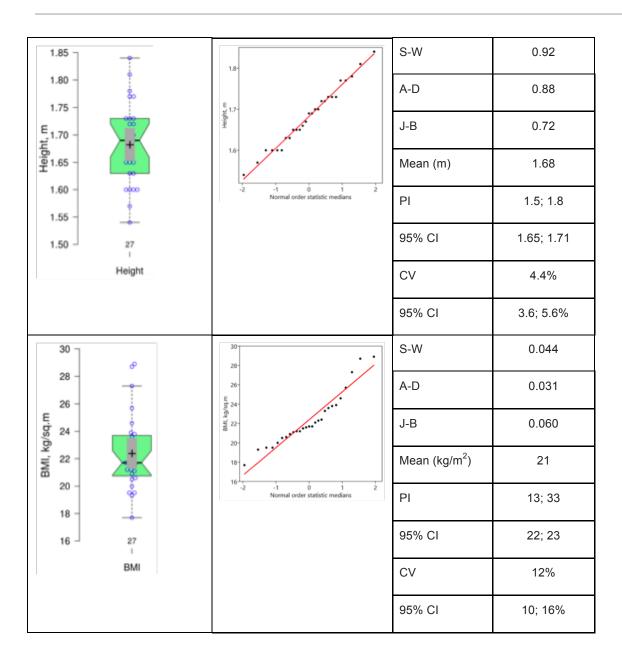
#### **Table 1.** Descriptive statistics of main anthropological characteristics

Box-and-whisker	Normal probability	Statistics	
40	40	Normality	<i>p</i> -values
35 -	35 30- 5 9 25- 20- 15- -2 -1 Normal order statistic medians	S-W	5•10 <sup>-4</sup>
30 - 30 - 30 - 30 - 40 - 15 - 27 - Age		A-D	2•10 <sup>-4</sup>
		J-B	0.037
		Mean (yr)	26
		PI	9; 36
		95% CI	23; 28
		CV	27%
		95% CI	22; 34%
90 J	90- 60- 50- 40- -2- Normal order statistic medians	S-W	0.26
80		A-D	0.17
- 07 Kg		J-B	0.063
<sup>8</sup> <sub>60</sub> −		Mean (kg)	64
50 - 40 - 27 ! Weight		PI	43; 110
		95% CI	60; 68
		CV	17%
		95% CI	12; 22%

### Accuracy of GoBe2<sup>™</sup> Smartband in Estimating the Calorie Intake of Food

S. Schaefer, PhD, Foods for Health Institute

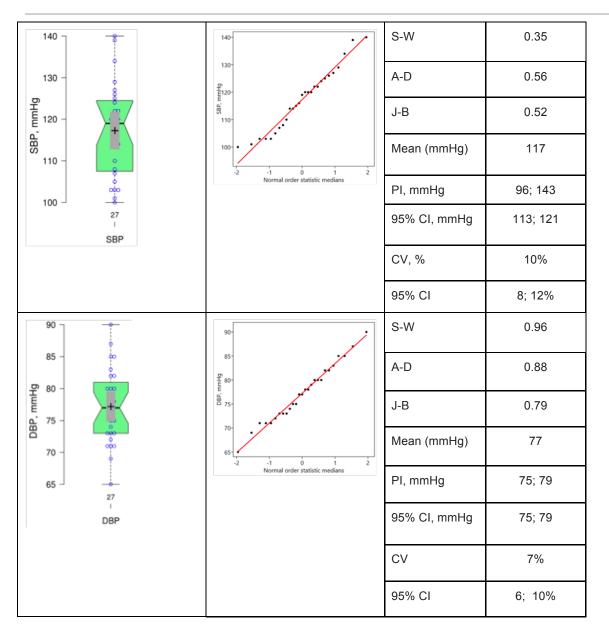
December 8, 2018



#### Accuracy of GoBe2<sup>™</sup> Smartband in Estimating the Calorie Intake of Food

S. Schaefer, PhD, Foods for Health Institute

December 8, 2018



Correlation analyses were conducted to examine relationships between the sample characteristics. Within the sample, correlations were observed between several measured variables, including body weight and blood pressure (Table 2).

#### Accuracy of GoBe2<sup>™</sup> Smartband in Estimating the Calorie Intake of Food

S. Schaefer, PhD, Foods for Health Institute

December 8, 2018

**Table 2.** Correlation analysis of the main anthropological characteristics of the study sample.

	Age	Weight	Height	BMI	SBP	DBP	Age Weight Height SBP DBP
		<i>p</i> -values				Weight 1 0.33	
Age		0.33	0.058	0.53	0.44	0.61	BMI 0.3 SBP
Weight	0.20		6•10 <sup>-4</sup>	10 <sup>-6</sup>	<b>4∙</b> 10 <sup>-5</sup>	0.0019	DBP
Height	0.37	0.61		0.53	0.0060	0.099	
BMI	0.12	0.79	0.13		0.012	0.015	
SBP	-0.15	0.71	0.51	0.47		<b>2∙</b> 10 <sup>-6</sup>	
DBP	0.10	0.57	0.32	0.46	0.77		
	Spearman's correlation coefficients, <i>r</i> s						

\*Values in bold are significant (*p*<0.05) or strongly correlated (*r*>0.70).

Analyses were conducted to compare calorie estimation in the GoBe2<sup>™</sup> compared to the reference method. Plots were constructed to examine how the two methods compared in their estimations of calorie intake (kcal/day) (Figure 2). The analyses were conducted using two sets of data: 1) Calorie (kcal/day) for all valid study days, and 2) Rolling averages (kcal/day) from 3-day intervals.

A paired sample test was conducted using the data set derived from all study days (kcal/day) and revealed a difference between the GoBe<sup>TM</sup> and reference method of less than a 200 kcal/day (p<0.0001). Spearman's rank order correlation showed a strong association between the two sample distributions ( $r_s$ =0.88, p<0.0001) (Table 3). When the same analyses were repeated with rolling averages, the methods were still different (p<0.0001) and Spearman's rank order correlation was not as strong ( $r_s$ =0.70) (Table 3).

### Accuracy of GoBe2<sup>™</sup> Smartband in Estimating the Calorie Intake of Food

S. Schaefer, PhD, Foods for Health Institute

December 8, 2018

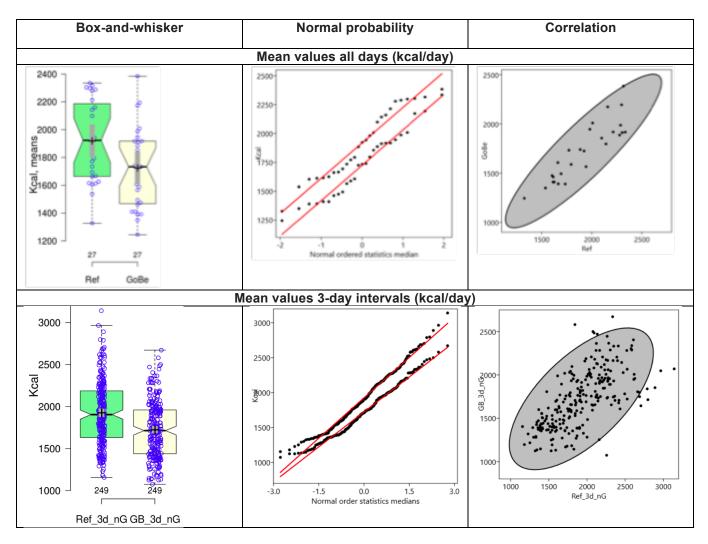


Figure 2. Mean values (kcal/day) for GoBe2<sup>™</sup> and reference method, all study days and 3-day intervals

Using the data from all study days, a regression model was created to predict the GoBe2<sup>™</sup>'s calorie intake (kcal/day) measurement as a linear function of the reference method (Figure 3). The model met the assumptions that the intercept did not deviate from the expected zero value, nor did the slope from the expected unit value.

A regression was also conducted with data by 3-day intervals which again resulted in a weaker correlation (Figure 4).

### Accuracy of GoBe2<sup>™</sup> Smartband in Estimating the Calorie Intake of Food

S. Schaefer, PhD, Foods for Health Institute

December 8, 2018

**Table 3.** Statistical comparisons of GoBe2<sup>™</sup> and reference method; kcal/day for all study days and by 3-day intervals

	All d	lays	3-day intervals		
	Reference	GoBe2™	Reference	GoBe2™	
Normality Tests	p-value				
S-W	0.07	0.39	0.01	0.004	
A-D	0.08	0.31	0.07	0.04	
J-B	0.24	0.44	0.05	0.009	
	Sta	atistics	I		
Mean (kcal/day)	1956	1778	1917	1724	
95% CI	1845, 2065	1660, 1890	1860, 1975	1680, 1770	
d	178		193		
95% CI	125-230		134-252		
RD	9%	6	10%		
95% CI	6%-12%		7% - 13%		
<i>p</i> -value	1.4•10 <sup>-7</sup>		10 <sup>-10</sup>		
<i>p</i> -value	1.4•10 <sup>-7</sup>		10	-10	

### Accuracy of GoBe2<sup>™</sup> Smartband in Estimating the Calorie Intake of Food

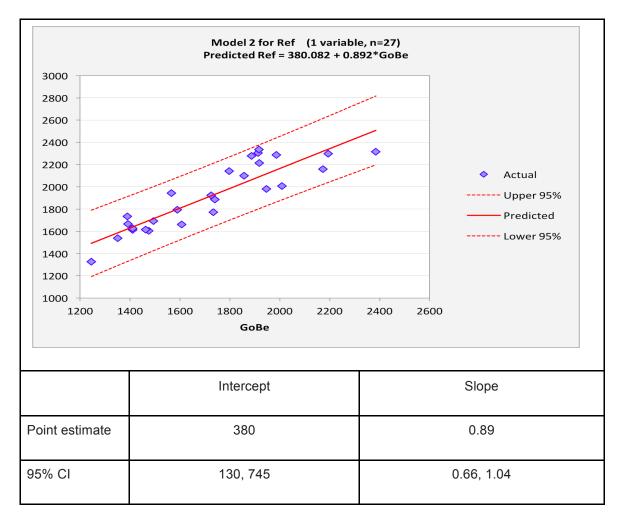
S. Schaefer, PhD, Foods for Health Institute

December 8, 2018

r <sub>S</sub>	0.88	0.70
95% CI	0.81-0.96	0.63-0.76
<i>p</i> -value	10 <sup>-9</sup>	10 <sup>-37</sup>

\*Normality tests: S-W = Shapiro-Wilk; A-D = Anderson-Darling; J-B = Jarque-Bera; RI = reference interval; PI = prediction interval; CI = confidence interval; d = mean difference; RD - relative difference;  $r_{S}$  - Spearman's rank order correlation

Figure 3. Regression model for predicting calorie intake measurement by GoBe2™ (all valid study days)



#### Accuracy of GoBe2<sup>™</sup> Smartband in Estimating the Calorie Intake of Food

S. Schaefer, PhD, Foods for Health Institute

December 8, 2018

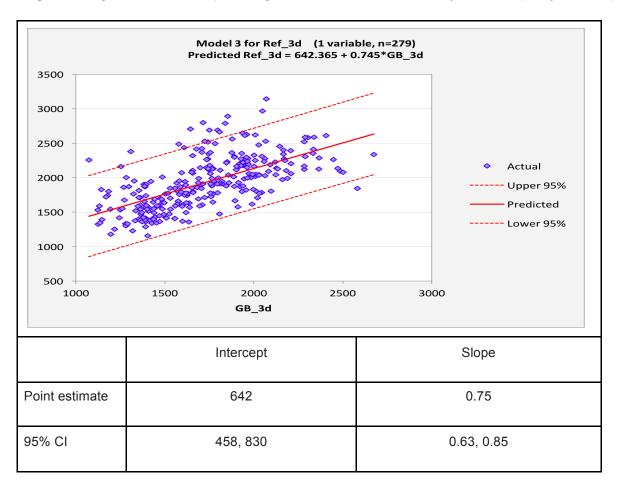


Figure 4. Regression model for predicting calorie intake measurement by GoBe2™ (3-day intervals)

### Limitations

This validation study was limited by several factors. The current form factor and battery life of the GoBe2<sup>™</sup> presented several barriers to consistent data collection, limiting its ability to accurately estimate calorie intake when not used properly. This was mitigated throughout the study by monitoring individual volunteers, developing tailored solutions when needed, and executing data cleaning processes to remove erroneous files. Other notable limitations were present within the reference method designed to validate the GoBe2<sup>™</sup>. Study volunteers were free-living, study methods utilized self-reporting methods to track food intake outside of the dining facility; however, reporting bias (misrepresenting food intake in dietary records) is noted in the scientific literature as a frequent source error in dietary reporting (Gemming 2016, Kipnis 2002, Scholler 1995). In attempt to mitigate reporting

#### Accuracy of GoBe2<sup>™</sup> Smartband in Estimating the Calorie Intake of Food

S. Schaefer, PhD, Foods for Health Institute

December 8, 2018

bias, several strategies were incorporated into the study design. First, volunteers were frequently encouraged to report all food intake by writing it on down on a record sheet, also checked in with research staff daily. Second, constantly monitoring blood glucose allowed staff to identify when blood glucose levels increased significantly, indicating food intake. If an such increase was not aligned with recorded food intake, these data were considered events of non-compliance and met exclusionary criteria for data analyses. Accurately measuring calorie intake also relied on the accuracy of nutritional information provided by all sources, including the USDA database and labels and packages from foods consumed by volunteers as part of the study, and also food that was considered "off protocol". When possible, volunteers were asked to confirm the actual weight (g) of any packaged food item with a digital scale, to the weight listed on the food package, as a measure to validate the nutritional information.

#### CONCLUSION

Healbe FLOW<sup>™</sup> technology calorie intake measurement by the GoBe2<sup>™</sup> provides good correlation compared to direct observation of intake in healthy adults; however, the current model of GoBe2<sup>™</sup> requires user training to avoid data loss.

#### REFERENCES

Center of Disease Control and Prevention. Defining Adult Overweight and Obesity. Accessed at <u>https://www.cdc.gov/obesity/adult/defining.html</u>

Dietary Guidelines for Americans, 2015-2020. United States Department of Agriculture. <u>https://www.choosemyplate.gov/</u>

Gemming L, Ni Mhurchu C. Dietary under-reporting: what foods and which meals are typically under-reported? Eur J Clin Nutr. 2016 May;70(5):640-1. doi: 10.1038/ejcn.2015.204. Epub 2015 Dec 16.

Kipnis V, Midthune D, Freedman L, Bingham S, Day NE, Riboli E, Ferrari P, Carroll RJ. Bias in dietary-report instruments and its implications for nutritional epidemiology. Public Health Nutr. 2002 Dec;5(6A):915-23.

Lohan TG, Roche AF, Martorell R. Anthropometric Standardization Reference Manual. Champaign: Human Kinetics Publishers; 1988.

Scholler DA.Limitations in the assessment of dietary energy intake by self-report. Metabolism. 1995 Feb;44(2 Suppl 2):18-22.