ABSTRACT

Background: Estimates of energy intake (EI) in humans have limited validity.

Objective: The objective was to test the accuracy and precision of the intake-balance method to estimate EI during weight gain induced by overfeeding.

Design: In 2 studies of controlled overfeeding (1 inpatient study and 1 outpatient study), baseline energy requirements were determined by a doubly labeled water study and caloric titration to weight maintenance. Overfeeding was prescribed as 140% of baseline energy requirements for 56 d. Changes in weight, fat mass (FM), and fat-free mass (FFM) were used to estimate change in energy stores (ΔES). Overfeeding EI was estimated as the sum of baseline energy requirements, thermic effect of food, and ΔES. The estimated overfeeding EI was then compared with the actual EI consumed in the metabolic chamber during the last week of overfeeding.

Results: In inpatient individuals, calculated EI during overfeeding determined from ΔES in FM and FFM was (mean ± SD) 3461 ± 848 kcal/d, which was not significantly (−29 ± 273 kcal/d or 0.8%); limits of agreement: −564, 505 kcal/d; P = 0.78) different from the actual EI provided (3490 ± 729 kcal/d). Estimated EI determined from ΔES in weight closely estimated actual intake (−7 ± 193 kcal/d or 0.2%; limits of agreement: −386, 370 kcal/d; P = 0.9).

In free-living individuals, estimated EI during overfeeding determined from ΔES in FM and FFM was 4123 ± 500 kcal/d and underestimated actual EI (4286 ± 488 kcal/d; −162 ± 301 kcal d or 3.8%; limits of agreement: −751, 427 kcal/d; P = 0.003). Estimated EI determined from ΔES in weight also underestimated actual intake (−159 ± 270 kcal/d or 3.7%; limits of agreement: −688, 370; P = 0.001).

Conclusion: The intake-balance method can be used to estimate EI during a period of weight gain as a result of 40% overfeeding in individuals who are inpatients or free-living with only a slight underestimate of actual EI by 0.2–3.8%. This trial was registered at clinicaltrials.gov as NCT 00565149 and NCT 01672632. Am J Clin Nutr doi: 10.3945/ajcn.114.087122.

INTRODUCTION

In 2011–2012, more than 78 million adult Americans were obese (1). Obesity prevalence continues to rise and is projected at 42% of adult Americans by the year 2050 (2). Obesity ensues from a sustained imbalance of energy whereby energy intake (EI) exceeds energy expenditure (EE), which results in an energy surplus stored as fat. Although the jury is still out on the cause(s) of this energy imbalance, there is increasing consensus that increased EI outweighs lower levels of physical activity, and thereby reduced EE is the most significant determinant of weight gain in most people (3, 4). Understanding the magnitude of the hyperphagia or overeating during periods of weight gain will help researchers to develop carefully controlled intervention studies and evidenced-based policy for obesity prevention through a reduction in dietary intake.

Assessing EI is a major shortcoming in obesity research in humans. For decades, scientific studies have relied on instruments that obtain estimates of EI from a self-report, in the form of food diaries or dietary recall. Although self-reported estimates of EI are somewhat acceptable in individuals with specific training such as dietitians (5), for the most part, the self-reported data show no differences in EI between BMI classifications (6), which, if viewed alone, would suggest that EI is not a contributing factor for weight gain.

In the early 1980s, the use of doubly labeled water (DLW) for the assessment of EE in free-living individuals was developed and validated (7). DLW is now considered the gold-standard method for estimating EI in weight-stable individuals. Comparisons of EI measured by DLW and self-report clearly indicated that individuals grossly underreport EI by an estimated 18% (8, 9), and this underreporting is more pronounced in overweight and obese individuals (10, 11). Despite this well-known finding, many investigators still rely on subjective instruments to assess EI. The study of food intake behavior during weight gain is hindered by the lack of accurate methods to quantify EI in free-living conditions (12), leaving the need for validated methods to estimate EI during weight gain. Several groups have demonstrated the validity and utility of the intake-balance method (EI =

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3Address correspondence to LM Redman, Pennington Biomedical Research Center, 6400 Perkins Road, Baton Rouge, LA 70808. E-mail: leanne.redman@pbrc.edu.
4Abbreviations used: DLW, doubly labeled water; DXA, dual-energy X-ray absorptiometry; EE, energy expenditure; EI, energy intake; ES, energy stores; FFM, fat-free mass; FM, fat mass; TEF, thermic effect of food.

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total daily EE + change in body energy stores (ES) to estimate EI in free-living individuals during weight stability or weight loss induced by dietary restriction (13–16), but a novel application of the intake-balance method is to estimate EI during weight gain induced by supervised overfeeding.

The aim of this work was therefore to assess the accuracy and precision of the intake-balance method to assess EI during weight stability or weight loss induced by dietary restriction (13–16), but a novel application of the intake-balance method is to estimate EI during weight gain induced by supervised overfeeding.

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energy X-ray absorptiometry (DXA) at baseline and then every other week during overfeeding.

Validation study II: weight gain induced by overfeeding in free-living participants

This study was approved by the Pennington Biomedical Institutional Review Board (www.clinicaltrials.gov; NCT 01672632). Volunteers provided written informed consent before participation.

Design

The second parent study used in this analysis was a prospective study to determine the effect of overfeeding on fat cell size and insulin sensitivity. Participants were provided with all meals during a baseline period (1 wk) and during the 56 d of overfeeding. They were outpatients, but they reported to the research kitchen during mealtimes where food was consumed under the supervision of a dietitian.

Participants

Thirty-five healthy, weight-stable individuals (29 men and 6 women) completed the study. Participants had to be aged 18–40 y and have a BMI between 22 and 32 at enrollment. Characteristics of the participants at baseline are shown in Table 1.

Determination of baseline energy requirements

Baseline energy requirements were calculated as the average between the estimates of EI from a prediction equation (19) and the EI consumed for weight maintenance during days 8–14 of a DLW study. During the first week of baseline and the DLW study (days 1–7), participants consumed a habitual diet in which the caloric intake was determined from a previously published equation for free-living, nonobese individuals (19). Beginning the second week (days 8–14), based on the total daily energy expenditure measured in days 1–7, EI was titrated up or down to weight stability with a diet consisting of 60% carbohydrate, 25% fat, and 15% protein.

Energy intake during overfeeding

The calorie intake of overfeeding was calculated as 140% of the baseline energy requirement. All meals were prepared by the metabolic kitchen and provided in a 5-d rotation with overfeeding kilocalories for all participants. The macronutrient composition of the study diet was identical to that in validation study I: 15% of energy from protein, 41% from carbohydrate, and 44% from fat (as analyzed by Covance Laboratories). Participants were instructed to consume all food provided. Additional food (including alcohol) was permitted and reported by participants during overfeeding.

Clinic assessments

Weight was measured at screening, daily for 7 d during energy balance, before overfeeding, and after overfeeding. Body composition was measured by DXA before overfeeding (baseline), at week 5 of overfeeding, and during the last week of overfeeding.

Description of outcome measures common to both trials

Twenty-four-hour energy expenditure and respiratory quotient by indirect room calorimetry

Twenty-four-hour metabolic chamber stays were completed at baseline and during the last week of overfeeding. Oxygen and carbon dioxide levels in the chambers were measured by using a Magnos 4G magneto-pneumatic oxygen analyzer and a Uras 3G infrared CO₂ analyzer (Hartmann and Braun), both of which sample O₂ and CO₂ concentrations 60 times per second. Every 10 s, a computer program averaged these values, calculated the volumes of O₂ consumption and CO₂ production, and plotted the average values at 10-min intervals. Energy expenditure and substrate oxidations (including respiratory quotient) were calculated from oxygen consumption, CO₂ production, and 24-h urinary nitrogen excretion by using the equations established by Acheson et al (20).

Total daily energy expenditure by DLW

Total daily energy expenditure was measured according to a standard procedure at Pennington Biomedical Research Center as previously described (19). In brief, after 2 baseline urine samples were collected, participants consumed a cocktail containing 0.2 g/kg of total water of ¹H₂¹⁸O (Cambridge Isotopes) and 0.115 g/kg of total body water of ²H₂O (Isotec Inc). Isotope elimination rates of ¹H and ¹⁸O (k₉ and k₈, respectively) were measured in urine collected at 4.5 and 6 h and then daily for the next 9 d (study 1) and on days 7 and 14 (study 2) by using linear regression based on the isotopic enrichment. Modifications of Schoeller (21) equations by Racette et al (22) were used to calculate carbon dioxide production. Total daily energy expenditure was calculated by multiplying the rate of carbon dioxide by the energy equivalent of carbon dioxide based on the measured respiratory quotient in the metabolic chamber.

Body composition

Body composition was measured by DXA by using the Hologic QDR 4500A whole-body scanner. The scans were analyzed with QDR software version 11.1 (Hologics).

Estimated energy intake calculation

The estimated EI during the overfeeding period was calculated according to the intake-balance method with the baseline energy requirement summed with the estimated thermic effect of food (TEF) of the overfeeding diet and changes in energy deposition in fat-free mass (FFM) and fat mass (FM).

First, to account for the increase in TEF with consumption of the overfeeding diet, we computed the overfeeding TEF as 10% of the overfeeding kilocalories for all participants.

Overfeeding TEF(kcal/d) = [actual overfeeding EI
- baseline energy requirement] × 10%     (1)

Second, the daily change in ES in FFM and FM was calculated from DXA and metabolic weight measurements and divided by the number of days of overfeeding. The coefficients used for tissue gain were assumed to be 13.1 kcal/g of FM gained and
2.2 kcal/g of FFM gained, which reflect the energy content of the tissue and the energy cost of tissue synthesis (4).

\[
\Delta ES_{\text{FM/FFM}}(\text{kcal/d}) = ([13.1 \text{ kcal} \times \Delta \text{FM(g/d)})] + ([2.2 \text{ kcal} \times \Delta \text{FFM(g/d)})]
\]  

(2)

Estimates of EI were then calculated as the sum of the baseline energy requirement, overfeeding TEF, and changes in body energy stores (\(\Delta ES\)) over the 56-d study.

Estimated EI(kal/d) = baseline energy requirement + overfeeding TEF + \(\Delta ES\)  

(3)

Estimated EI (kcal/d) was then compared with the actual EI provided (kcal/d) during overfeeding. The actual EI includes kilocalories attributed to foods returned to the kitchen (weigh back) or, in the case of the free-living individuals, additional foods consumed as reported by the participants.

Although 7.4 kcal/g is a commonly used energy coefficient for weight (4), estimation of the change in energy storage from weight change requires an assumption of energy partitioning. To account for this, we calculated an energy coefficient for each participant:

\[
\Delta \text{ES}\text{weight}(\text{kcal/d}) = 8.4 \text{ kcal} \times \Delta \text{weight(g/d)}
\]  

(4)

The average energy coefficient for weight across the participants, 8.4 kcal/g, also was used to estimate EI from the change in energy stores computed from the daily change in metabolic weight.

\[
\Delta \text{ES}\text{weight}(\text{kcal/d}) = 8.4 \text{ kcal} \times \Delta \text{weight(g/d)}
\]  

(5)

Given that 7.4 kcal/g is a commonly used energy coefficient for \(\Delta \text{ES}\text{weight}\), estimated EI was also calculated by using 7.4 kcal/g as the energy coefficient, and the results can be found in Supplementary Table 1 and Supplementary Figure 1 (under “Supplementary data” in the online issue).

Statistical analysis

All calculations and data analysis were performed by a biostatistician (HH) with SAS version 9.3 (SAS Institute). The Student’s \(t\) test for paired samples along with Bland-Altman regression analysis (23, 24) were used to determine differences between estimated and actual EI. The Student’s \(t\) test for paired samples was used to evaluate the validity of estimated EI compared with actual EI during overfeeding. Bland-Altman regression analysis was used to evaluate whether the mean difference varied across different amounts of actual EI (proportional bias). Data are reported as means ± SDs, and \(P < 0.05\) was considered significant.

RESULTS

Validation study I: estimated EI with weight gain induced by overfeeding participants on a metabolic ward

Baseline characteristics

Although sex was balanced (5 men and 3 women), the participants were predominantly black. The mean BMI was 26.0 ± 2.9 and ranged from 22.2 to 29.6 (Table 1). The average weight gained during overfeeding was 6.3 ± 2.4 kg but varied considerably among the participants (4.1–11.0 kg). Most (56.0 ± 13.7%) of the excess weight was deposited in FM (3.5 ± 1.6 kg), but the FFM was also expanded (2.8 ± 1.5 kg).

Calorie intake and EE

EI required for baseline weight maintenance was 2443 ± 510 kcal/d and was significantly higher than total daily EE measured at baseline by DLW (2146 ± 557 kcal/d, \(P = 0.036\)). The actual average EI consumed during overfeeding was 3490 ± 729 kcal/d. The overfeeding diet thereby provided an additional 1027 ± 190 kcal/d or 139.5% of the baseline energy requirement.

Validation of estimating EI during overfeeding

The mean baseline energy requirement (2443 ± 510 kcal/d), when summed with the overfeeding TEF (105 ± 23 kcal/d) and \(\Delta \text{ES}\) from FFM and FM (913 ± 395 kcal/d), derived an estimated EI of 3461 ± 848 kcal/d during overfeeding (Table 2). Estimated EI during overfeeding by using \(\Delta \text{ES}\) from FFM and FM was not significantly different from actual intake during overfeeding (−29 ± 273 kcal/d or 0.8%; \(P = 0.78\); limits of agreement: −564, 505 kcal/d). The Bland-Altman plot shows (Figure 1A) that the proportional bias was not significant across different amounts of actual intake (\(P = 0.27\)).

Estimated EI during overfeeding by using \(\Delta \text{ES}\) from weight was not significantly different from actual intake during overfeeding (−7 ± 193 kcal/d or 0.2%; limits of agreement: −386, 370 kcal/d; \(P = 0.9\)). The proportional bias was significant across different amounts of actual intake (\(P = 0.03\); Figure 1B).

Validation study II: Overfeeding and weight gain in free-living participants

Baseline characteristics

This study population was predominantly male and white. The mean BMI was 25.6 ± 2.3 and ranged from 22.5 to 31.4 (Table 1). The average weight gained during overfeeding was 7.2 ± 1.9 kg but ranged considerably between the participants (2.3–10.7 kg). Similar to the inpatient study (validation study I), most (57.2 ± 14.8%) of the excess weight was stored in FM (4.1 ± 1.3 kg; 0.8–7 kg), but the FFM was also expanded (3.1 ± 1.4 kg; 0.5–6 kg).

Calorie intake and EE

The baseline energy requirement was 3046 ± 393 kcal/d and was not significantly different from the total daily EE measured during baseline by DLW (3054 ± 567 kcal/d, \(P = 0.42\)). The actual average EI provided during overfeeding was 4286 ± 488 kcal/d. This provided an additional 1240 ± 170 kcal/d during overfeeding or 140.3% of the baseline energy requirement.

Validation of estimating EI during overfeeding

As shown in Table 2, the mean baseline energy requirement (3046 ± 393 kcal/d), when summed with the TEF associated with overfeeding (124 ± 17 kcal/d) and \(\Delta \text{ES}\) from FFM and FM (954 ± 286 kcal/d), derived an estimated EI during overfeeding of 4123 ± 500 kcal/d. The estimated EI using \(\Delta \text{ES}\) from FFM and FM significantly (\(P = 0.003\)) underestimated actual EI.
TABLE 2
Calculations of EI

<table>
<thead>
<tr>
<th></th>
<th>Validation study I (inpatient; n = 8)</th>
<th>Validation study II (outpatient; n = 35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline energy requirement (kcal/d)</td>
<td>2443 ± 510 (1906–3310)</td>
<td>3046 ± 393 (2186–4078)</td>
</tr>
<tr>
<td>Actual overfeeding EI (kcal/d)</td>
<td>3490 ± 729 (2760–4694)</td>
<td>4286 ± 488 (3130–5183)</td>
</tr>
<tr>
<td>TEF (kcal/d)</td>
<td>105 ± 23 (85–138)</td>
<td>124 ± 17 (86–157.2)</td>
</tr>
<tr>
<td>ΔWeight (kg)</td>
<td>6.3 ± 2.4 (4.1–11.0)</td>
<td>7.2 ± 1.9 (2.3–10.7)</td>
</tr>
<tr>
<td>ΔESweight (kcal/d)</td>
<td>935 ± 371 (604–1644)</td>
<td>957 ± 259 (312–1428)</td>
</tr>
<tr>
<td>ΔFM (kg)</td>
<td>3.5 ± 1.6 (2.3–7.2)</td>
<td>4.1 ± 1.3 (0.8–7.0)</td>
</tr>
<tr>
<td>ΔFFM (kg)</td>
<td>2.8 ± 1.5 (0.9–5.7)</td>
<td>3.1 ± 1.4 (0.5–6.0)</td>
</tr>
<tr>
<td>ΔESFFM/FM (kcal/d)</td>
<td>913 ± 395 (646–1830)</td>
<td>954 ± 286 (215–1537)</td>
</tr>
<tr>
<td>Estimated overfeeding EI (kcal/d) From ΔESFFM/FM</td>
<td>3461 ± 848 (2754–4973)</td>
<td>4123 ± 500* (3017–5454)</td>
</tr>
<tr>
<td>From ΔESweight</td>
<td>3482 ± 874 (2596–4837)</td>
<td>4126 ± 492* (3244–5409)</td>
</tr>
</tbody>
</table>

1 Values are means ± SDs; ranges in parentheses. Student’s t test was used for paired samples to evaluate the validity of estimated EI compared with actual EI during overfeeding. *P < 0.05. EI, energy intake; ES, energy stores; FFM, fat-free mass; FM, fat mass; TEF, thermic effect of food; Δ, change from baseline to day 56 of overfeeding.

DISCUSSION

In this report, we used data derived in a landmark overfeeding study in which participants resided on a metabolic ward for 56 d and consumed 40% above baseline energy requirements (17). In this controlled feeding study, participants consumed all meals under supervision and consumed only foods that were prepared by the metabolic kitchen. The EI calculated from the intake-balance method underestimated actual EI by <1%. The use of weight alone to estimate the change in energy stores also underestimated actual EI but with improved accuracy and precision compared with estimated EI from FM and FFM.

In addition, we used data derived from an overfeeding study in which participants, while consuming foods provided by the metabolic kitchen, were free-living throughout the trial. Even in free-living individuals, the intake-balance method estimated EI within 4% of actual EI consumed. Although estimated EI in free-living individuals is statistically less than actual EI during overfeeding, the intake-balance method provides a far more accurate and objective assessment of EI than many self-report instruments, which underestimate actual EI by 15–20% (8, 9).

We assumed that estimated EI of individuals with minimal and repeatable levels of physical activity (and hence EE) on a metabolic ward would be more accurate than those derived in free-living participants with variable activity. Although EI estimates cannot be directly compared because no 2 participants completed both studies, the data support this hypothesis. The EI

![Figure 1](https://example.com/figure1.png)

**FIGURE 1.** Comparison of actual EI consumed compared with EI calculated by the intake-balance method by using changes in energy stores from fat mass/fat-free mass (A) and body weight (B) in participants (n = 8) confined to a metabolic ward during a controlled overfeeding study for 56 d. Black circles indicate females, and gray circles indicate males. The dashed lines represent the mean and the mean ± 1.96 SD. A: The proportional bias was not significant across different amounts of actual intake (P = 0.27). B: The proportional bias was significant across different amounts of actual intake (P = 0.03). EI, energy intake.
measured by the intake-balance method more closely approximated food provision during overfeeding in those who were confined to a metabolic ward compared with individuals who were free-living.

Although 40% overfeeding is greater than what the general weight-gaining population consumes habitually, the use of clinical data from short-term inpatient and outpatient studies of 40% overfeeding allows a unique opportunity for researchers to test the effect of weight gain in a well-controlled environment during a short period that would more realistically occur over 6–12 mo. The analysis also indicates that if body weight is the only available measure for estimating changes in ES, estimates of EI can be likely derived within 4% of actual EI consumed, which again outperforms any assessment of EI from self-report (8). In our previous work in dietary restriction, we learned that point estimates of EI can be derived by using the intake-balance method (DLW and DXA) over both shorter (1 mo) and longer (3, 6, or 12 mo) durations (13, 22). This study in overfeeding, which supports the use of the intake-balance method in the same fashion, is a valuable tool for researchers and clinicians to objectively assess EI in their patients at 6- or 12-mo follow-up visits more accurately than self-report assessments, resulting in more reliable research findings and more effective weight management recommendations.

Even though we used gold-standard assessments for estimating the baseline energy requirement and changes in ES, the intake-balance method was not able to account for all calories consumed during the 2 independent overfeeding studies. The intake-balance method indeed outperforms any self-reported instrument. However, there are some limitations to consider, many of which probably involve inherent measurement error. For example, the DLW method, although the most accurate method for quantifying total daily EE in free-living individuals, has a CV of 5–7% in comparison with the gold-standard measure of EE by room calorimetry, which has a CV of 2–3% (21). It is important to note, however, that we did not just rely on the total daily EE measurement from the DLW study to determine the baseline energy requirement. Instead, we used a hybrid of approaches, but most important, the estimated energy requirement was tested for its ability to maintain weight stability in the participants at baseline over several days. Recognizing that many groups will not have the capability for DLW, it is noted that baseline energy requirements can also be calculated from numerous prediction equations for total daily EE (19, 25) or resting metabolic rate multiplied by an activity factor (26). The accuracy and precision of these methods have been tested for different populations and ethnic groups (27–30).

Although the observed difference between the EI estimated by the intake-balance method and the EI provided is small, some of this discrepancy might be explained by 2 assumptions made in the calculation of EI from the intake-balance method. Without a DLW study during the overfeeding period, the total daily EE component of our equation required some assumptions. First, the increased TEF of the overfeeding diets (≈140% of baseline energy requirements) was assumed to be 10% for all participants but likely varied slightly between individuals. Second, in using the baseline energy requirement, which is the EI for weight stability, including habitual activity (either inpatient in study 1 or outpatient in study 2), we assumed that the level of physical activity of the participants was maintained throughout the overfeeding period. Perhaps an actual measure of total daily EE might be argued to have provided a more accurate and precise estimation of EI in our study, but an improvement greater than 1–4% would be unlikely. This is owed to the importance of establishing the baseline energy requirement for weight stability in these kinds of studies (31). Our previous work in weight loss suggested that limitations for estimating EI are affected by the precision of measurements used to estimate changes in ES (13). For example, the repeatability of DXA to assess percentage of body fat is 1% (32). Therefore, when DXA is used to estimate changes in ES, a change <1% may be due not to true changes in energy stores but to measurement error. Although we are confident there were measureable changes in FM and FFM in all participants, our measurements, and thus calculation of

![FIGURE 2. Comparison of actual EI consumed compared with EI calculated by the intake-balance method by using changes in energy stores from fat mass/fat-free mass (A) and body weight (B) in free-living participants (n = 35) during a controlled overfeeding study for 56 d. Black circles indicate females, and gray circles indicate males. The dashed lines represent the mean and the mean ± 1.96 SD. The proportional bias was not significant across different amounts of actual intake (A: P = 0.70; B: P = 0.65). EI, energy intake.](image)
estimated EI during overfeeding, is limited by the precision of the DXA.

It remains a goal of scientists to develop methods that can accurately estimate habitual EI in humans and with potential for widespread scalability. The cost of feeding individuals to energy balance obviously limits the use of the intake-balance method in clinical weight management programs. The recent development and validation of mathematical models from a culmination of studies with gold-standard measures of energy requirements (by DLW or caloric titration) and body composition (by DXA), however, allow estimates of EI in free-living individuals to be more widely available (33–35). These models, centered on the intake-balance method and theoretical estimates of changes in energy stores, have been conveniently extrapolated to user-friendly Web-based platforms and therefore have the potential to be used in clinical settings to provide data-driven counseling to patients on EI.

In summary, the intake-balance method can provide an accurate, precise, and objective assessment of EI during weight gain. This objective measure allows for a more truthful look at the causes of obesity and provides us with evidence-based clinical tools to treat this widespread epidemic.

We are indebted to Susan Mancuso for study coordination for both of these challenging study protocols, Courtney Brock as the research kitchen director, Jennifer Rood for mass spectroscopy oversight, and of course the study participants for making this body of work possible. The authors’ responsibilities were as follows—LMR, ER, and GAB: designed this study; HH: conducted the analysis; LAG, LMR, and ER: interpreted the data; and LAG and LMR: prepared the manuscript. All authors signed this study; HH: conducted the analysis; LAG, LMR, and ER: participants for making this body of work possible.

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