Dear Sir:

We read with interest the validation study for ferritin in capillary blood by Ahluwalia et al (1). As pointed out by the authors, there is a need for “tools that are applicable for field use in remote areas.” Subsequent to the publication of the spot ferritin assay for samples dried on filter paper (2), we used this method to measure serum ferritin in a survey conducted in a very isolated indigenous group in northern Mexico (3) after adapting the method for use under field conditions (4). We would like to share some of the difficulties encountered when using this technique and reflect on its potential for use in remote settings.

First, although the filter-paper method avoids the need to freeze samples, samples still need to be centrifuged. In our survey, we ran a microcentrifuge from a car battery with the use of a converter to transform 12-V direct current to 110-V alternating current. Transporting and using this equipment (weighing up to 25 kg) can be difficult in isolated locations. Second, breaking the microcapillary tube to extract the serum exactly between the verter to transform 12-V direct current to 110-V alternating current.

We (1) recently developed a filter-paper method for measuring hemoglobin with a HemoCue (HemoCue, Inc, Mission Viejo, CA), and the last 3–4 drops are used to fill a 75-µL microcapillary tube. The amount of serum available diminishes as the hemoglobin concentration increases; thus, in persons with adequate iron status, a larger volume might be needed to provide the 20 µL serum required for the analysis. A finger prick is often considered less invasive and more acceptable than is venipuncture. Although this could be true when obtaining 2 drops of blood, it might not be so if 6 or more drops are taken. Many of the women we surveyed stated that finger-prick sampling was more painful than was having venous blood drawn.

In conclusion, specialized and relatively expensive equipment and well-trained personnel are needed to use the ferritin assay for capillary samples spotted on filter paper. These factors need to be taken into account when planning surveys in remote and isolated field settings.

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Reply to J Monárrez-Espino and T Greiner

Dear Sir:

We (1) recently developed a filter-paper method for measuring serum ferritin and validated its use in young children in a field setting in Sri Lanka (2). Monárrez-Espino et al (3) measured serum ferritin with the use of our filter paper–based dried serum spot (DSS) ferritin assay (1) and measured hemoglobin with the HemoCue method (HemoCue, Inc, Mission Viejo, CA) in young indigenous women in northern Mexico and showed that anemia was mainly due to iron deficiency (3). In their letter, Monárrez-Espino and Greiner share their experience from that survey in a “very isolated” indigenous group in northern Mexico and raise certain issues pertaining to the process of collecting blood, obtaining serum, and preparing DSS samples on filter paper. We will address the points raised in this order.

Blood sampling, whether venous or capillary, involves some degree of discomfort. Capillary blood sampling offers the advantage of being more acceptable to certain groups, such as young infants, children, and the elderly, and may be the only option available...
in certain cultures. Capillary blood can be collected by trained field technicians rather than by para-health professionals, who are usually required for venous blood collection. The training of field technicians to collect capillary blood samples and to separate serum is simple, but necessary, and requires a minimal amount of time (typically 1 d). In the Sri Lankan study (2), a trained laboratory technician collected the finger-prick samples into ≥2 capillary tubes (75 mm each). Thus, ≥150 μL blood was typically collected and provided more than an adequate volume of serum necessary for the DSS preparation.

From our experience in Sri Lanka (2) and Guatemala (N Ahluwalia, J Bulux J, NW Solomons, unpublished observations, 2001) and that of others (4), whole blood ferritin is neither a reliable nor a valid tool in which to assess body iron stores, and results obtained from plasma ferritin vary (5). Thus, serum must be used for measuring ferritin to assess iron status. In our experience, when capillary tubes with an internal diameter of 1 or 1.5 mm are used for obtaining serum, the percentage of tubes that are rendered unusable is small.

Monárrez-Espino and Greiner present the difficulties experienced with the transport and use of a microcentrifuge in their survey in a “very isolated” community where electricity was not available. In our experience in Sri Lanka (2) and Guatemala (N Ahluwalia et al, unpublished observations, 2001), we did not encounter these difficulties. The samples were transported to the laboratory and centrifuged within 1–1.5 h of collection in Sri Lanka and centrifuged within 1–1.5 h of collection directly in the field with a microcentrifuge (weight: 10 kg) in Guatemala. In a highly remote setting where electricity is not available, some adaptations to centrifuging blood samples are necessary.

In our study with Sri Lankan children (2), the technician scored and broke off the spun capillary tubes without spillage after only minimal training. However, to address and potentially overcome this step—in collaboration with investigators from the Center for Studies of Sensory Impairment, Aging and Metabolism (Guatemala)—we recently finished a study in periurban Guatemala in which we used a special pipette and dispenser that make use of the blood clot itself to push a fixed volume of 20 μL serum directly from the capillary tube. The preliminary results of this study are promising (6).

Finally, in the Guatemalan study, we also evaluated other field user-friendly techniques, such as cutting out a circle with a diameter equivalent to 20 μL serum from the DSS and measuring ferritin using our spot assay (2). We found that the DSS (blot and cut) approach provided ferritin values that did not differ significantly from values obtained from serum samples stored frozen until analyzed with the traditional method (6). We plan to publish the results of this study in the coming year. The cost of the pipette (range: $65–$235) or the special dispenser ($200) is a one-time cost only and most laboratories may already have this simple equipment.

In conclusion, the spot ferritin assay is based on DSS samples that can be prepared from either venous or capillary blood on filter paper by fieldworkers with minimal, but essential, training with the use of readily available or easily obtainable equipment. The advantages of this method need to be kept in mind when considering its use. These advantages include the lack of need for a cold chain, the stability of ferritin in DSS samples stored at room temperature for up to 1 mo, the ease of shipping DSS samples, and the reduced likelihood of transmission of certain blood-borne pathogens.

Enhanced lipogenesis after fasting

Dear Sir:

Wyatt and Hill (1) in their editorial about the excellent article by Weinsier et al (2) ask “whether it requires more physical activity to prevent weight regain than to prevent weight gain.” In the 1960s, Harry Antoniades and I conducted a series of studies involving insulin and a protein with a higher molecular weight that we called bound insulin, which now, I believe, is called insulin-like growth factor. We extracted and partially characterized bound insulin from human serum and from a rat liver perfusate to which we had added insulin. In one of our studies with Huber et al (3), we reported that the epididymal fat pads of rats deprived of food for 4 d and then refed ad libitum showed a marked increase in lipogenesis compared with the fat pads from control rats in the presence of glucose and either insulin or bound insulin. Although we only reported data after 7 d of refeeding, I recall observing enhanced lipogenesis in the rats refed for 20 d. I wrote in our article, “It would seem reasonable to ask whether the rapid accumulation of fat often observed in people after a period of dieting might not in part be the result of increased ability to synthesize fat.” This might answer, at least in part, the questions raised by Wyatt and Hill.
physical activity, increased energy intake, or both. If, in fact, metabolic or physiologic differences exist between gainers and maintainers, the role that these differences play are likely to be largely expressed through behavior.
They estimated that this difference in activity energy expenditure accounted for 77% of the excess positive energy balance in the gainers.

We concur with Wyatt and Hill’s (2) endorsement of the need for rejuvenating the public health promotion of physical activity. However, several additional methodologic issues and limitations of this study should be considered.

The study sample consisted of 2 very different groups of women—those who were at their normal body weight at baseline and those who had recently lost ≥10 kg in response to an energy-restricted diet. Not surprisingly, 19 of the 20 gainers were formerly overweight or obese, whereas only 6 of the 27 maintainers were formerly overweight or obese. A better study design would have been one in which the study subjects were derived from the same population, because there may be important behavioral or metabolic differences between persons with and without a recent history of significant weight loss.

On the basis of their data, Weinsier et al suggest that ~80 min/d spent on activities of daily living of light-to-moderate intensity, 2- to 4-fold higher than current physical activity recommendations (3, 4), are necessary to prevent weight gain. However, they provided no information on the quantity or quality of physical activity during the follow-up period. Actual data concerning the quantity and quality of physical activity while the subjects were free-living during the 1 y of follow-up would be helpful in evaluating this assertion. How much more walking, stair climbing, and other activities of daily living were the maintainers doing relative to the gainers?

Finally, Weinsier et al suggest that physical activity is more important than is diet in the control of body weight, but they provided no data on the dietary habits of the subjects before weight loss and during the follow-up period. It is important to underscore that there were no significant changes over time in any component of energy expenditure in either group of women; the statistically significant findings remained limited to cross-sectional comparisons. Weinsier et al seem to draw the puzzling inference that the formerly overweight or obese women reduced their weight by dietary means, yet regained their weight solely as a result of low physical activity. Given that energy expenditure did not change in these inactive women, how can this inference be valid? The other half of the energy-balance equation, ie, energy intake and dietary quality during the follow-up period, certainly may have influenced weight regain.

At present, the best approach to preventing obesity and chronic disease appears to be through comprehensive lifestyle modification (5, 6), although the ideal dietary and physical activity prescriptions to control body weight over the long term remain elusive.

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Reply to MA Pereira et al

Dear Sir:

We wish to respond to many issues that Pereira et al raised regarding the methods used in our recently published study (1). First, Pereira et al described our study sample as consisting of “2 very different groups of women” and suggested that a better study design would be one in which the subjects were derived from the same population. The reason provided for this assertion was that there may be important behavioral or metabolic differences between persons with and without a recent history of weight loss.

Our intent in selecting both previously overweight and previously normal-weight subjects was to obtain a baseline study group that would give a wide range of weight-gain responses during follow-up. In fact, as was outlined in our article, the subjects were phenotypically homogeneous, ie, percentage body fat; metabolic characteristics of resting energy expenditure, fuel utilization, or insulin sensitivity; activity energy expenditure (AEE); and physical activity (activity-related time equivalent index) were not significantly different between the 2 groups of women. Consequently, we viewed our subjects as one group that was likely to vary widely in the propensity toward obesity on the basis of their weight histories. For example, some of the previously normal-weight women were likely to gain weight over time, simply because they were still young and had not yet had a chance to gain much weight; 14% of the 28 women recruited with no history of overweight did not maintain their baseline weight status over the year. By contrast, whereas some of the weight-reduced women were likely to gain a significant amount of weight, this was not the case for all of them. This was evidenced by the fact that, whereas 95% of the “gainers” were previously overweight women, only 58% of the previously overweight women were gainers. Thus, although the subjects should have provided a range of weight-gain responses enabling investigation of factors relating to weight gain and
weight maintenance, they could not be considered 2 distinct
groups in terms of an a priori assumption that they would or would
not gain weight.

We agree with the rationale that there may be important behav-
ioral or metabolic differences between having or not having a
recent history of weight loss, and our data support this notion. We
did not report dietary intake patterns in this article, and we are
not convinced that such reports would be as accurate or as objec-
tive as were our measures of sleeping energy expenditure (SEE)
and AEE, which were measured by chamber calorimetry and the
doubly labeled water method, respectively. Our study showed no
significant differences in patterns of SEE, fuel utilization, or exer-
cise economy between the “maintainers” and the gainers. Fur-
thermore, because we had reasonably good measures of body
composition, we were able to calculate the energy cost associated
with accumulating the weight gained by the gainers over the year.
The difference in energy expended in physical activity between
the gainers and maintainers accounted for 77% of the energy cost
of weight gain. Given that SEE did not change, we concluded that
physical activity has a strong influence on variations in relative
weight and adiposity. If our calculations are correct, then AEE
appeared to play the major role in the weight gain and would have
been more important in this group of subjects than even dietary
intake. Luke et al (2) recently confirmed that AEE is strongly
associated with adiposity and, with age, sex, and weight,
explained 77% of the variance in percentage body fat. However,
despite the importance of AEE, we certainly do not discount the
role that dietary intake plays in altered weight status.

Finally, we agree with the suggestion by Pereira et al that
evaluating the nature of differences in physical activity (inten-
sity, duration, and frequency) would be valuable in better
understanding the role that physical activity and exercise play
in preventing weight gain. To this end, we have undertaken a
study to evaluate the influence of aerobic and resistance train-
ing on weight maintenance after weight loss and the nature of
self-selected, free-living physical activity displayed by gain-
ers and maintainers. Unfortunately, we did not have good
descriptors for the nature of the activity patterns of the sub-
jects in our completed study (1).

Finally, a point raised by Pereira et al regarding the interpretation
of our statistical analyses needs clarification. In repeated-measures
analyses of longitudinal data, the analyses are not reduced to a
cross-sectional comparison if the measured variable does not
change over time. In fact, cross-sectional analyses do not lend
themselves to deductions about cause and effect relations. In our
study, wherein the gainers had lower levels of physical activity
and fitness than did the maintainers, in both their normal- and
weight-gain states, the data indicate clearly that weight gain per
se was not the cause of the lower physical activities or fitness lev-
els. Conversely, the data suggest that lower activities and fitness
levels may well have contributed to the weight gain observed in
our subjects.

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Erratum

Abrams SA, Griffin IJ, Davilla PM. Calcium and zinc absorption from lactose-containing and lactose-free infant

On page 443 (column 2, fourth and fifth paragraphs), the doses of 70Zn, 67Zn, and 46Ca should be in micrograms,
and the carbohydrate content of both formulas should be 73.3 g/L in Table 1.